membered, however, that the topical application of steroids may precipitate an attack of herpes simplex keratitis. Several cases so induced, some with disastrous end-results, have been reported. Adequate long-term controlled studies are still needed before the value of combined antibiotic-steroid therapy in trachoma can be assessed. In the meantime, its routine use in mass campaigns cannot be justified.

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Thrombosis and Vascular Disease: A Review of Research Needs *

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Several WHO Expert Committees have, in the past, considered broad aspects of vascular disease in man. The present authors were invited to consider, in the light of present-day knowledge, the interrelationship of thrombosis and vascular disease and to recommend how the problem may be studied. The main body of this report outlines how this can be done.

"Thrombosis" may be defined as the accretion of material from the circulating blood on the surface of a vessel leading to a varying degree of interference with blood flow. The thrombus may in time become organized and even completely incorporated into the vessel wall or it may be broken up within minutes or days, leaving no trace. In the initial stages a thrombus is made up of varying proportions of platelets, fibrin, erythrocytes, leucocytes, other blood constituents and perhaps material derived from the vessel wall. Platelets and fibrin are considered to be the most important constituents.

"Coagulation" can be defined as the formation of fibrin from fibrinogen by a complex interaction of factors—a process which may take place within the vessel lumen, in the tissues or in the blood *in vitro*. While coagulation has an important role to play in the process of thrombus formation, it must not be regarded as a term synonymous with thrombosis.

The structure of thrombi varies considerably. Arterial thrombi formed in a high-flow pulsatile system may show striking differences from those developing in the venous system where blood flow is slow and sometimes static; at least in the former, platelets are an important and probably necessary constituent. Further, the term "thrombus" covers a range of meanings, from the transient or fleeting accretion of solid material on the vessel wall to that which completely occludes the vessel with cessation of blood flow.

The factors to be considered in any study of the etiology and pathogenesis of thrombosis can be classified broadly as characteristics of: (*a*) the blood vessel wall, (*b*) the blood flow, (*c*) the circulating blood.

Although a great many studies have been made of each of these factors, their exact interrelationship during the evolution of vascular disease has not been defined and therefore requires further study. Several

* This report was drafted as a result of discussions organized by the World Health Organization with a view to designating the areas in which research on thrombosis and vascular disease is urgently needed and to indicating in which of those areas international co-ordination might be of the greatest benefit. The Cardiovascular Diseases unit of the World Health Organization, Palais des Nations, Geneva, Switzerland, welcomes comments and suggestions from research workers and institutes interested in this problem.—ED.

recent advances have been made and the resulting techniques can now be applied along with established techniques in experimental, clinical, therapeutic, or population studies. Material for these investigations can be obtained from: (*a*) living individuals, (*b*) autopsies on humans, (*c*) animal experiments.

RECENT ADVANCES

It is clearly important that recent information pertaining to the following be taken into account before future studies are considered:

(1) The effect of adenosine nucleotides on platelets;

(2) Electron microscopic studies of thrombi and vessel walls;

(3) The role of factor XIII (fibrin stabilizing factor) in fibrin formation;

(4) Enzyme studies of the vessel wall;

(5) The production of thrombi in experimental animals;

(6) Radioisotope techniques, as applied to the study of platelets and plasma proteins;

(7) Microcirculation studies, as applied to investigations on vessels, blood constituents and haemodynamics.

SUGGESTED METHODS OF STUDY

For clarity we have listed the available techniques in relation to the vessel, blood flow, and blood constituents. Some techniques provide information relevant to more than one of these aspects. Since the objective is to study thrombosis and vascular disease, investigation of any factor in the blood must be related to thrombosis and vascular disease and frequently animal experiment is the only way in which this can be done.

I. *Studies in living individuals*

A. Blood vessel walls

1. *In situ* study of microcirculation in the nail bed, conjunctiva, retina and skin.

2. *In vivo* platelet adhesion to damaged vessel walls.

3. Bleeding intensity and bleeding time.

4. Cine-angiography.

5. Study of vessels in tissues removed at operation.

6. Study of diseased vessels removed during vascular surgery.

B. Blood flow

1. Cine-angiography.

2. Study of microcirculation (see A.1 above).

C. Circulating blood

1. Determination of fibrinogen levels (factor I).

2. Determination of fibrin stabilizing factor (factor XIII).

3. Study of fibrinogen survival and turnover with radioactive isotopes.

4. Determination of fibrinolytic activity in plasma, serum (or fractions), or whole blood, in combination with various activators or substrates, and evaluation of inhibitors.

5. Determination of the presence or absence of fibrin-fibrinogen degradation products.

6. Determination of factors related to contact activation (factors XI and XII) and related activity, e.g., serum thrombotic accelerator activity of Wessler.

7. Assay of other individual coagulation factors (II, V, VII, VIII, IX, X).

8. Estimation of inhibitors of blood coagulation:
   (*a*) antithrombin;
   (*b*) antithromboplastin;
   (*c*) others.

9. Platelets:
   (*a*) platelet count;
   (*b*) platelet adhesiveness;
   (*c*) platelet aggregation and deaggregation;
   (*d*) platelet survival and turnover using isotope techniques;
   (*e*) platelet biochemistry.

10. Assessment of heparin inhibition.

11. Factors as measured in A.2 and A.3 above.

12. Comprehensive (global) tests of coagulation, e.g., thrombelastography of Hartert.

13. Other factors:
   (*a*) erythrocytes
      (i) adenosine diphosphate (ADP) content and release,
      (ii) thromboplastic component,
      (iii) haematocrit;
   (*b*) leucocytes;
   (*c*) lipids
      (i) phospholipids,
      (ii) triglycerides,

(iii) cholesterol,
(iv) cholesterol esters,
(v) non-esterified fatty acids;
(*d*) lipoproteins and related factors such as plasma lipase activity (lipoprotein lipase);
(*e*) muco-proteins;
(*f*) catecholamines;
(*g*) other hormones.

II. *Human autopsy studies* (including surgical material specified in I.A.5 and I.A.6)

A. Blood vessel and contents

1. Gross inspection and localization of lesions.

2. Quantitative measurement of various aspects of the vessel wall disease and of thrombi.

3. Histological study of vessel wall and thrombi by:

(*a*) light and electron microscopy;
(*b*) histochemistry;
(*c*) immunochemistry.

4. Biochemical and functional characteristics of the vessel wall and contents including large and small thrombi.

(*a*) quantitative chemical analysis of various components, e.g.,

(i) lipids,
(ii) collagen,
(iii) calcium,
(iv) mucopolysaccharides,
(v) adenosine nucleotides such as adenosine diphosphate,
(vi) structures such as mitochondria;

(*b*) enzyme and other activity, e.g.,

(i) fibrinolytic activity,
(ii) thromboplastic activity,
(iii) phosphatase and other activity which could lead to change in ADP,
(iv) glycolysis and oxygen uptake.

B. Blood flow

The relationship of any abnormal configuration of the vessel wall to the location of thrombi should be studied, with particular reference to distortion caused by disease or congenital abnormality, in order to define more accurately their possible effect on the localization of the thrombi (see I.A.4 and I.B.1 above).

C. Blood

The specific recommendations with regard to the study of the blood are included under II.A. above. It must be kept in mind that as thrombi can break up after death and before autopsy is performed, it is often difficult to determine the role of thrombosis in a particular event. A technique must be evolved to investigate this problem. Also, methods should be explored for the study of the cadaver blood.

III. *Animal experiments*

There are several new and old experimental systems that have been developed to study the problem of thrombosis in animals. These include:

(*a*) dietary experiments;
(*b*) injury experiments;
(*c*) stasis experiments;
(*d*) *in vivo* and *in vitro* shunts;
(*e*) microcirculation experiments;
(*f*) injections of material of known or suspected thrombus-promoting activity.

The techniques that can be used in these studies are similar to those listed above for living individuals. Species differences should also be examined.

GROUP STUDIES

The following group studies, organized on an international basis where appropriate, would provide essential information.

I. *A prospective study in a population with a known high risk of thrombosis and in which a high percentage of autopsies could be done*

The objective of this survey would be to evaluate the ability of certain procedures to predict in any population those persons liable to the clinical complications of vascular disease and their relationship to thrombosis.

The size of the selected group should be large enough for a significant number of deaths—e.g., 200—to occur within a two-year period, and it is envisaged that a population of 20 000 persons between the ages of 40 and 60 years would have to be examined. (These figures are illustrative of the magnitude of the study as we envisage it.) Further, it might be necessary to continue the survey at yearly intervals for a period of five years or longer to determine whether the factors governing the prediction might be a variable feature in any individual. In the selection of persons to be included in this survey, as many variables as possible in terms of

social status, occupation, habits and environment, should be included. It may be advisable or expedient to divide the group into several subgroups. Such a study should be under the guidance of an epidemiologist and a statistical expert who could advise on this and other points and define the size of the sample.

If a multi-centre study is decided upon, there should be central control of methods and rigid standardization of apparatus. All reagents, glassware, needles, etc. should be supplied from a central source.

The details of techniques used to study the blood and vessels should be decided upon by those involved in the trial but they should in broad outline measure:

(*a*) platelet function (see I.C.9; page 553),

(*b*) fibrin and fibrinolysis (see I.C.1, 2, 3, 4 and 5).

II. *Comparative group studies on living persons*

These should be done:

(*a*) between populations with a known low and high risk of thrombosis, e.g., Uganda and North America;

(*b*) in stable ethnic groups with different characteristics;

(*c*) in ethnic groups where a proportion is undergoing environmental change due to the impact of a different civilization or following immigration, e.g., Africans adopting a European way of life and diet, or Asians in the USA, etc.

The initial objective of these studies is to determine whether differences exist. The tests used should in broad outline be those already described but these could be of limited scope depending on the facilities available. Group numbers may not need to be large. All ages of these populations should be part of the survey and autopsies should be included where possible. When this phase of the investigation has been completed the reasons for differences or lack thereof must be explored.

In this and other studies which involve the clinical evaluation of the subjects, the methods of assessment must be carefully standardized. Where possible, the suggestions of the appropriate WHO expert groups should be followed. In the analysis of much of the data from these studies, computer facilities may be required and the advantages of setting up a central unit for this purpose should be investigated.

III. *Survey of special diseases*

A very useful step would be to institute worldwide collection of data on the incidence of thrombosis, extent of vascular disease and characteristics of the blood in the undermentioned specific and often rare conditions. In addition, suitable autopsy and surgical specimens should be collected from these and where possible from appropriate control groups for study by all of the methods listed under "Suggested methods of study" in section II.A. The exact details of the protocol and material will have to be worked out and standardized by the participating groups and will vary depending upon the exact nature of the problem being studied; e.g., the protocol and material needed for a study of peripheral venous thrombosis will be quite different from those required for an electron microscopic study of vessel walls in diabetics.

A. Haemorrhagic diseases

1. Congenital or acquired, isolated or combined, deficiency of coagulation factors with particular reference to factors:

I (congenital afibrinogenaemia),
VIII (haemophilia A),
IX (Christmas disease, haemophilia B or PTC deficiency),
XI (PTA deficiency),
XII (Hageman factor deficiency),
XIII (fibrin stabilizing factor deficiency).

2. Von Willebrand's syndrome.

3. Platelet abnormalities such as thrombocytopathia or thrombasthenia.

B. Other abnormalities of the blood

1. Myeloproliferative diseases:

(*a*) polycythaemia vera;
(*b*) myelofibrosis;
(*c*) chronic myeloid leukaemia;
(*d*) thrombocytosis.

2. Chronic haemolytic anaemia with special reference to sickle cell disease.

C. Other diseases

Diabetes	Hypothyroidism
Hypertension	Hyperlipidaemias
Cirrhosis of the liver	Mitral stenosis
Peptic ulcer	Brain tumour
Chronic alcoholism	Carcinoma of lung

Steatorrhoea or tropical sprue

D. Other conditions

The frequency of thrombosis in the female during the reproductive period with reference to the menstrual cycle, pregnancy and the *post partum*

period. This should include where possible a detailed study of the uterine vessels.

IV. *Intensive study of selected groups*

A. Families with an apparent inherited tendency to an increased incidence of thrombosis

There has been very little work done on the genetic aspects of thrombosis and it is possible that careful study of these families may provide information on this important problem. It is felt, indeed, that, because of its importance, more attention must be paid to the question of genetics and thrombosis.

B. Persons who by accepted data would be expected to suffer from thrombosis, e.g., individuals with hyperlipidaemia, but who have no clinical evidence of advanced vascular disease

V. *Studies on patients with clinical evidence of vascular disease*

While these groups have already been widely investigated and differences established between them and age-paired controls, there is a need to apply some of the newer techniques to this problem. The differences observed in this type of study should be evaluated in prospective studies.

VI. *Animal studies*

A. Spontaneous thrombosis

This has been reported in old dogs, cats and some other animals. Arrangements might usefully be made for the collection of this information on an international scale. In addition an attempt should be made to locate, breed and maintain animals with congenital defects of coagulation. These animals can be used for the study of the disease and in experimental situations. The establishment of such colonies should be encouraged and a central registry of information organized.

B. Experimental studies

Experimental studies should be initiated where appropriate to supplement the information from human observation.

VII. *Other studies suitable for international co-ordination*

A. Studies of the effect of environment and habits on the incidence of thrombosis

1. Climate.
2. Temperature.
3. Altitude.
4. Soil chemistry.
5. Water supply.
6. Exercise.
7. Diet.
8. Smoking, including types of tobacco and method of smoking.
9. Alcohol.

B. Geographic studies of the origin and habitat of persons dying from the complications of vascular disease in selected countries or cities

These might cover, for instance, two adjacent cities with similar environments but different water supplies.

C. Study of the geographic incidence of varicose veins and of venous thrombosis

PROPHYLAXIS AND TREATMENT OF THROMBOSIS

The assessment of measures for the prevention or treatment of thrombosis must be carried out in properly designed trials. In view of the fact that we are dealing with chronic disease processes this is of great importance.

I. *Mono- and di-coumarin and indanedione derivatives and heparin and heparin-like substances*

The use of these drugs in the management of both acute and chronic arterial disease remains controversial. In many groups of individuals treated with anticoagulants the amounts of the drugs administered have not been sufficient to produce a continuous marked modification of the coagulation mechanism. Results of animal experiments suggest that use of inadequate amounts of these drugs does not prevent thrombosis and may even be thrombogenic. Therefore there is a need for further carefully controlled trials of these anticoagulants using effective dosage, both in the treatment of established thrombosis and in the prevention of thrombosis in persons considered to be at risk. These trials should be carried out in properly selected groups with adequate controls and laboratory control of those coagulation factors thought to be involved in thrombosis.

It is important in studies of this nature to carry out specific detailed autopsy examinations along the lines already indicated to assess whether there are any differences in thrombosis and vessel wall disease between the groups. This could be organized in multiple centres under the aegis of a suitable international body.

It should be pointed out that there is still need for detailed study of the metabolism of the anticoagulant drugs, particularly in relation to their effects on thrombosis.

II. *Fibrinolysis*

Further research is urgently required into:

(*a*) the local or systemic therapy of thrombosis by lysis induced *in vivo* with activators of the system, and

(*b*) the prophylactic induction by various agents of mild *in vivo* lysis over a prolonged period.

III. *Diet*

There is evidence that the occurrence of thrombosis in the experimental animal can be influenced by dietary fat. In human studies designed to assess the prophylactic effect of diet on vascular disease and its complications, attention should be paid to changes in the characteristics of blood coagulation and thrombus formation and to autopsy studies conducted along the lines indicated for the other investigations.

IV. *Other factors*

Any other agents employed for the prevention of vascular disease and its complications should be evaluated in a similar manner.

CONCLUSIONS

We have sought to present our views of the overall problem of arterial and venous thrombosis and its relationship to disease of the vessels and abnormalities of the circulating blood. Although an indication of the priorities to be given in respect of the various trials and methods of study discussed might be of value, we do not feel that, in the present state of knowledge, we can give a compelling reason for selecting any one aspect for particular study. Systematic examination of the whole problem in perspective would seem to be the most certain way in which the maximum understanding of this complex subject can be achieved.

Existing laboratories and research groups will continue to investigate their own individual problems. These might be asked to consider the proposals in this report and to participate or to co-operate in international studies by providing advice, by training research personnel or by providing selected laboratory facilities for the examination of material collected elsewhere. Similarly, an approach might be made to individuals who are in a position to contribute to an international study by participating or by providing source material. The collection of the data suggested in sections I, II and III above, under " Suggested methods of study ", will in many instances depend on the protocols decided upon by the participating groups. In other situations where the information required is as to the existence and availability of material, either human or non-human, this could be collected without delay. The large-scale studies of the predictive value of specific techniques should be initiated as soon as possible following the assessment of the staff facilities, and selection of the appropriate methods and techniques for the participating group or groups.

Further research is required before recommendations can be made with any certainty as to the exact techniques that should be used in studies of living or dead individuals. However, to allow a start to be made to international studies without delay, tests measuring the following factors might be carried out as being most likely to produce information of value:

(*a*) fibrinogen levels,

(*b*) fibrinolytic activity (I.C.4 under " Suggested methods of study " above),

(*c*) platelet function (I.C.9),

(*d*) comprehensive tests of coagulation (I.C.12),

(*e*) studies of vessel wall and thrombi (I.A. and II).

The final decision on the exact techniques to be used for each particular aspect must be left to those who take part in the studies.

All the autopsy studies recommended could be carried out immediately by selected laboratories that have skills and facilities encompassing all the techniques to be used. When necessary, materials from other centres could be sent to these laboratories.

Finally, thrombosis and vascular disease constitute one of the major causes of morbidity and mortality in the world today and this problem is still far from clearly understood. An urgent need exists for some central organization to bring together those working in the different disciplines involved in order to discuss plans for future co-ordinated research.