# THE CENTRAL LABORATORY
## OF THE NETHERLANDS RED CROSS BLOOD TRANSFUSION SERVICE

(Director: Dr. J. J. van Loghem)

The Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, the origin of which dates from 1939, is guided by a foundation in which participate the Dutch Government, the Netherlands Red Cross and the Municipality of Amsterdam.

A staff of physicians, chemists, pharmacists and biologists is charged with the leading of the different departments and laboratories. The Central Laboratory which is operating on a non-profit basis, is the medico-technical centre of the blood transfusion organization in the Netherlands.

It provides the majority of Dutch hospitals with blood transfusion equipment and blood derivatives. Besides the large-scale investigations in blood grouping and antibody screening in pregnant women and Red Cross donors, research work is done on various subjects covering the large field of blood transfusion, immunohematology, immunopathology, immunochemistry, biochemistry of proteins and blood clotting.

The Central Laboratory comprises 4 producing departments and 9 laboratories. [1]

The producing departments are in charge of :

1. **The production of sterile, pyrogenfree transfusion equipment and infusion fluids.** — The taking and giving sets are made of disposable plastic material. About 260 hospitals are regularly provided with blood transfusion material. The production of

[1] *Plate.* (Photographs L. M. Tangel, The Hague.)

transfusion equipment increases with the growing number of blood transfusions. At the present time about 180,000 blood transfusions are given annually in the Netherlands (40,000 hospital beds).

Utmost care is given to prevent untoward reactions due to pyrogens or bacterial contaminants.

2. **Freeze-drying of blood plasma, plasma fractions and human milk.** — The blood for the preparation of plasma and plasma fractions is collected by mobile teams. Yearly 40,000 to 50,000 bottles of citrated blood are used. (The donors for the procurement of blood plasma form a separate group of Red Cross donors ; they do not spend their blood for direct transfusion of whole blood in hospitals.)

Plasma is prepared from fresh human blood, that is to say, within 12 hours after taking, the blood is centrifuged by means of Laval centrifuges ; a second centrifugation is performed in a turbine centrifuge (Sharples). The clear liquid plasma is irradiated, frozen and lyophilized.

The plasma is prepared from pools containing not more than twelve separate donations, to minimize the risk of transmitting homologous serum jaundice. Human plasma is used for stockpiling and is further applied in cases where whole blood is not immediately available for the prevention and treatment of shock. For the treatment of burns human plasma is indicated.

Part of the remaining blood cells are used for the preparations of washed red cells suspensions, white cell-poor blood or purified platelet suspensions for patients who need this special treatment.

3. **Fractionation of plasma proteins.** — The plasma proteins are separated following the aethanol fractionation method developed by Cohn and slightly modified in the Central Laboratory. The raw protein fractions are isolated and purified following the scheme presented below :

**Plasma** from citrated blood :

Ethanol concentration 8 pCt. pH 7.2
temp. —3°C. ionic strength 0,14

precipitate : fibrinogen (+ anti-hemophilic factor).

Ethanol concentration 25 pCt pH 6.9
temp. —5°C. ionic strength 0,09
precipitate : $\gamma$-, $\beta$-globulin, prothrombin

Ethanol concentration 40 pCt. pH 5.8
temp. —5°C. ionic strength 0,09
precipitate : $\alpha$- and $\beta$-globulin

Ethanol doncentration 40 pCt pH 4.8
temp. —5°C. ionic strength 0,11
precipitate : albumin.

A recent development in the field of plasma protein fractionation is the preparation of a pasteurized plasma protein solution, containing albumin, $\alpha$- and $\beta$-globulin, which is equivalent to normal plasma as far as the colloid-osmotic pressure is concerned, and which has the advantage over plasma that there in no risk of transmitting homologous serum jaundice. Human albumin, a preparation of the protein component which forms about 80 % of the total protein content of the plasma of whole human blood, is used for the treatment of shock and in cases of hypalbuminemia.

Human gamma globulin, a preparation of the plasma proteins, prepared from whole human blood containing the antibodies of normal adults, is obtained from pooled liquid human plasma from not less than 1000 donors. This 'normal' gamma globulin is used for the prevention of infectious diseases (measles, infectious hepatitis, chicken-pox and mumps).

Normal gamma globulin is also used for the treatment of bacterial infections frequently occurring in patients with a- or hypogammaglobulinemia.

Besides this, gamma globulin is produced from the plasma of rubella convalescents for the prevention of rubella in pregnant women and antivaccina gamma globulin prepared from the blood of adults shortly after smallpox vaccination. This last product is applied in the Army to prevent encephalitis postvaccinalis after primovaccination.

Human fibrinogen, a dried preparation of the soluble constituent of liquid human plasma which, on the addition of thrombin is transformed to fibrin, is used in cases of fibrinogenopenia and fibrinolysis.

Fibrinogen with anti-hemophilic factor, prepared from fresh plasma, is used to produce a temporary correction of the deficiency of the anti-hemophilic factor in hemophilia.

Fibrin foam, prepared from fibrinogen by the addition of thrombin, is usually applied in combination with thrombin in case of hemorrhages.

4. **The production of blood typing reagents.** — The majority of blood typing reagents is prepared from human blood. The test sera anti-A, anti-B and anti-A + -B are prepared from human blood with a high titre of anti-A and anti-B antibodies (mainly obtained from the Military Blood Transfusion Service).

The great majority of anti-Rh and other sera with immune antibodies is obtained from subjects immunized by pregnancy or blood transfusion. Anti-M and anti-N sera are generally prepared in rabbits, but sometimes from human serum with naturally occurring antibodies.

Anti human globulin serum is prepared in rabbits.

It has also been possible to prepare useful blood grouping reagents from extracts of certain seeds, especially anti-N from seed extracts of Vicia Graminea.

A special laboratory is in charge of the control of all products prepared in this institute. All preparations meet the minimum requirements laid down in the protocol of the European Agreement on the Exchange of Therapeutic Substances of Human Origin (Council of Europe).

## Laboratory investigations

1a. **Blood typing of pregnant women.** — In 1952 the Dutch Government initiated the prenatal Rh testing of all pregnant women. This is organized in the following way :

In the 17 regional laboratories of Public Health, ABO and Rh (D) typing is performed of pregnant women in the third month of

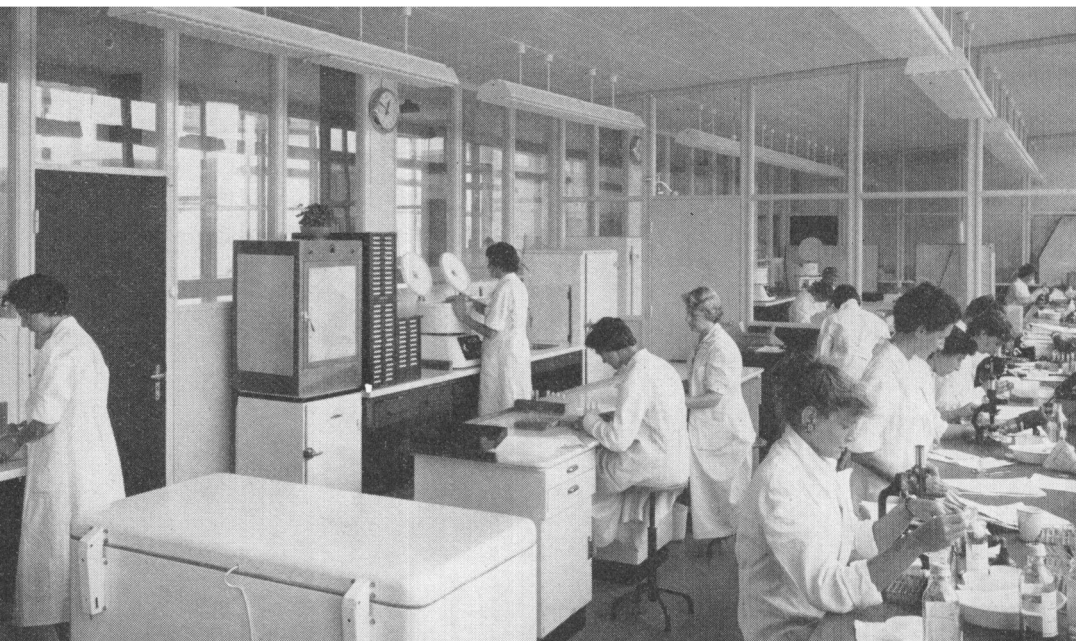
*Main entrance*

*AMSTERDAM.* — The Central Laboratory of the Netherlands Red Cross Blood Transfusion Service.

*Filling the blood-transfusion bottles with anti-coagulation fluids.*

*Drying of blood plasma.*

*Laboratories for the research of bloodgroups.*



pregnancy. At the same time are done the serological tests for syphilis.

The blood of all Rh negative (D-negative) women is sent to the Central Institutes for Blood Group Research for the investigations of Rh antibodies. For the three northern provinces the blood is sent to the Serological Laboratory at Groningen, for the 8 other provinces the blood is sent to the Central Laboratory at Amsterdam.

The purposes of this arrangement are :

*a)* to reduce the lethality from hemolytic disease in the newborn and to minimize the risk of cerebral damage and other sequelae, by adequate therapy (exchange transfusions);

*b)* to prevent Rh negative women from being immunized by Rh positive blood transfusion.

The organization of systematic prenatal Rh testing has greatly contributed to the prevention and adequate treatment of hemolytic disease in the newborn. Rh antibodies are found in about 4 % of the Rh negative pregnant women. The number of cases of Rh sensitization detected annually in the Central Laboratory amounts to about 700.

1b. **Blood typing of Red Cross donors.** — ABO and D-typing of the Red Cross donors is done in the regional laboratories. The blood of the D negative donors is sent to the Central Institutes for Blood Group Research, where additional investigations are performed for the C, E and $D^u$ antigen.

D negative, but C- or E positive subjects are marked as Rh positive donors, but as Rh negative recipients.

This service is also maintained by the Government.

2. **Serological studies in immunization by pregnancy or blood transfusion.** — Apart from the prenatally detected cases of Rh sensitization, a great number of cases suspected of hemolytic disease is studied. These are due to Rh sensitization or ABO-immunization; a minor percentage is caused by Rh antibodies other than anti-D, such as anti-o, anti-E, anti-e, anti-$C^w$ or other immune antibodies (anti-Kell, anti-$Fy^a$).

Irregular antibodies in the serum of patients or donors are analysed. Serological studies are made in cases of transfusion reactions and cross-matching problems.

A large cell panel of fresh and frozen cells carrying all known blood group antigens in different combinations is maintained for the study of specific iso- and auto-antibodies.

3. **Serological studies in hemolytic anemia and allied diseases.** — A large number of blood samples of patients suffering from hemolytic anemia is continuously under investigation. The various types of auto-antibodies against red cells are analysed and eventually the specificity is determined.

Most of the auto-antibodies belong to the immune type and are of the incomplete variety. Besides this a number of patients with high-titred cold agglutinins and various types of auto-hemolysis have been observed.

4. **Antibodies against white cells and platelets.**

*a)* **Iso-antibodies.** — Studies on white cell and platelet iso-antibodies are performed in relation to blood transfusion reactions and immunization during pregnancy. A great number of febrile reactions and chills occurring after multiple transfusions are due to the occurrence of white cell antibodies, especially of the incomplete form.

These reactions can be prevented by the administration of white cell-poor blood.

Also platelet antibodies are sometimes the result of repeated blood transfusion, though their occurrence is far less frequent than white cell antibodies.

*b)* **Auto-antibodies.** — Auto-antibodies against white cells and platelets are mainly of the incomplete variety and may be a cause of increased destruction of these cellular elements.

Platelet antibodies are found in nearly 50 % of patients suffering from idiopathic thrombopenia, and in lower percentage also in secondary thrombopenia, associated with lupus erythematodes diffusus and other blood diseases.

The occurrence of auto-antibodies against antigens in the cytoplasma of white cells is very rare. They are sometimes observed in

patients with idiopathic leucopenia, panmyelophthisis and other hemopathias.

In the blood of all patients suffering from lupus erythematodes diffusus auto-antibodies against nuclear antigens are present. They are responsible for the formation of LE cells.

Much attention is given to the development of appropriate methods for the recognition of these types of antibodies.

5. **Auto-antibodies in other diseases.** — The recognition of antibodies against other autologous antigens is a subject of research, such as the detection of sperm antibodies in patients with a- or oligozoospermia, and auto-antibodies against thyreoglobulin in patients with thyroiditis, etc.

6. **Serological reactions in rheumatic diseases** are performed on a large scale.

Different methods are applied (AST, Rose test and other appropriate techniques).

7. **Blood group genetics and sero-anthropology.** — Genetical studies of blood groups are applied in cases of disputed paternity, exchange of babies, linkage with hereditary diseases, etc.

Mass blood typing of ABO and D factors of the Army in the Netherlands revealed significant differences in the blood group distribution between the population in large towns and countrymen.

Detailed sero-anthropological studies were made of other populations, such as aborigines of N. Guinea, Surinam, Iran, French Basques, and the African Goldcoast.

8. **Biochemical and immunochemical studies.** — The introduction of biochemical methods in addition to the serological investigations in patients suffering from blood diseases is of great help in the study of auto-immunization.

In general the following determinations are done :

*a)* estimation of haptoglobin values ; low hemoglobin levels are found in patients with increased red cell destruction ;

*b)* electrophoretic studies of serum proteins ; particularly the Oughterlony method, the immuno-electrophoretic technique of

Grabar and Williams, and the analysis of serum proteins by means of the ultracentrifuge have refined the study of serum proteins and antibodies ;

*c)* detection of abnormal hemoglobins by means of chromatography on Amberlite-columns, electrophoresis and alkali-denaturation ;

*d)* estimation of the survival rate of red cells and platelets with radioactive tracers ($Cr^{51}$) to determine the site of cell destruction (liver and spleen) ;

*e)* the survival of serum proteins can be estimated with $J^{131}$, which is of practical importance in patients with hypo- and agammaglobulinemia.

In the field of immunochemistry attention is given to the determination of complement fractions by the kinetic method, and other serum factors (e.g. properdin) of importance in immunology.

9. **Blood clotting.** — Blood transfusion therapy is applied in many patients with disorders in the blood clotting mechanism. Sometimes more or less purified blood products are applied as therapeutic substances, anti hemophilic globulin for the treatment of hemophilia A and fibrinogen in large quantities in patients with afibrinogenemia.

Since 1954 studies are made in patients with disorders of the blood clotting mechanism; complete analysis can be made of the clotting factors.

## Educational programme

Blood group courses are regularly given for the education of laboratory technicians. A special laboratory has been reserved for continuous practical training of technicians working in the Dutch hospital laboratories.

In cooperation with the Military Blood Transfusion Service courses are given to medical and auxiliary personnel of the Army on the various aspects of blood transfusion therapy.

Since 1945 more than 2000 medical officers and 2300 other members of the Army Medical Service followed these courses.

## International relations

The Central Laboratory is associated with several international organizations, such as the International Society of Blood Transfusion and the League of Red Cross Societies, and as far as standardization of blood transfusion equipment and exchange of blood products is concerned, with the International Organization for Standardization, the Council of Europe and the World Health Organization, the latter especially in regard to international scientific and educational programmes.