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"BIOMED": INTRODUCTION OF A NEW TECHNOLOGY IN BLOOD FRACTIONATION

DP/MON/87/003

MONGOLIA

Technical report: Consolidation of the Blood Fractionation Unit and introduction of a new technology*

Prepared for the Government of the Mongolian People's Republic
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Mr. Ludmil G. Bozadjiev, expert in blood fractionation,
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United Nations Industrial Development Organization
Vienna

* This document has not been edited.

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ABSTRACT

Programme "Biomed" Consolidation of the Blood Fractionation Unit and introduction of a new technology

The examination of the equipment supplied in the accomplishment of the project DP/MON/82 showed that some items were not put into action. Under my supervision the Sartocon ultrafiltration module and the lyophilisation apparatus Usifroid were put into action.

The examination of the supplied equipment for the implementation of the new project showed that some essential items were not timely delivered.

Despite these difficulties the needed conditions were created to process 180 l retroplacental serum for the isolation of a "raw" immunoglobulin for intravenous use by a new unknown technology in Mongolia . This technology was applied also in the fractionation of 80 l retroplacental serum which had higher concentration of anti-staphyloccocal antibodies.

The ion exchange chromatography stage was carried out on a pilot scale twice and the production of high purity immunoglobulin for intravenous use was demonstrated. The second procedure was carried out by the local specialists themselves under my guidance.

A programme for the theoretical and practical training of local personnel on the methods of isolation of immunoglobulin for intravenous use was strictly followed.

INTRODUCTION

The implementation of the Project DP/MON/32 of the UNIDO Programme "Biomed" at the Blood Fractionation Unit of the Institute of Bioproducts (now Institute of Hygiene) resulted in the creation of production and technological facilities for the processing of 3000 l retroplacental serum per year. Suitable up-to-date technologies were introduced in the immunoglobulin and albumin production which now meet the WHO requirements on quality.

By means of the new technologies and equipment the production capacity of the Blood fractionation Unit was increased.

The future development with the support of the UNIDO project DP/MON/87/003 would result in an immunoglobulin preparation for intravenous use. To this end new technology will be introduced.

The authors duties were to

-give continuous attention to the theoretical and practical training of local personnel in the technological processes in blood fractionation;

-lecture and perform experimental work on new developments in the production of blood derivative - special attention to be paid to polyethylenglycol fractionation and ionexchange chromatography;

-perform on-site training of local personnel in production of immunoglobulin preparations for intravenous use from placental sources;

-evaluate the work on the project and make recommendations on future research and production of blood derivatives.

These duties were fulfilled as described further on.

ACTIVITIES CARRIED OUT

The author's mission started with examining the available equipment, done in cooperation with the UNIDO Maintenance Engineer. This examination showed that some essential items were not used or were not put into action (the ultrafiltration module Sartocon, the freeze-drying apparatus "Usifroid", the Pharmacia system for gel filtration). The other items of the equipment supplied for the implementation of the Project DP/MON/82 were in good state and worked correctly.

The examination of the supplied equipment for the implementation of the new project showed that some essential items were not delivered at that time (Column KS 370, Preparative fraction collector, Pump R 100, Pressure vessel etc.).

At the beginning of the mission we found out that some of the trained personnel had left and new persons without experience and knowledge in the field of blood fractionation were appointed to fill the vacant positions. This fact presented a complimentary obstacle in the accomplishment of our work.

In cooperation with the maintenance engineer the author started working on the installation of the Cryofuge 5000-Heraeus Sepathech and translated into Russian the operating instruction of this equipment.

The Mongolian side proposed that the Sartocon ultrafiltration module for the diafiltration and concentration of the albumin solutions be put into action again under my supervision. The technology introduced in the fractionation of retroplacental serum for the production of human serum albumin was performed on the site again and the diafiltration and concentration of the product were introduced correctly (Annex 1). Local specialists were trained to work with this apparatus and it could be concluded that those engaged in this activity are capable of unsupervised work.

The lyophilization apparatus Usifroid after a small successful repair work carried out under the guidance and with the participation of the maintenance engineer was put into action. Two batches of immunoglobulin were freeze-dried successfully.

The author began the theoretical training of the workers following a programme which was prepared in advance.

Despite of the inavailability of some essential appatuses the needed condidtions were created to process under my direct guidance 180 l retroplacental serum and 80 l retroplacental serum which had higher concentration of anti-staphyloccocal antibodies for the isolation of "raw" immunoglobulins for intravenous use by fractionation with ethanol and polyethylenglycol.

In the production of "raw" immunoglobulins for intravenous use new, unknown technology in Mongolia was introduced (Annex 2). This technology ensured the removal of the immuno-

globulin aggregates from the retroplacental serum and these could achieve a good technological practice in the control of the parameters.

In the course of the production the equipment supplied for the implementation of the project DP/MON/82 was used.

The job training for the local specialists and workers was carried out both theoretically and practically on the application of the new technology and the correct work with the equipment. (Annex 4).

Detailed description of the technology was prepared. The needed amounts of reagents and materials were calculated and the technological instruction was handed over to the Mongolian specialists.

The second step of the technological process, the ionexchange chromatorgaphy was carried out on a pilot scale because of the delay in the delivery of the production column and preparative fraction collector.

Under my direct guidance the needed conditions were created to process the raw immunoglobulin with a small column filled with 0,5 kg of CM-Sepharose CL-6B supplied by myself.

New, unknown in Mongolia ionexchange chromatography method for the production of highly purified immunoglobulin for intravenous use was demonstrated (Annex 3) and on-the-job training for the local specialists and workers was carried out both theoretically and practically. The application of ionexchange chromatography, the activation and regeneration of the ionexchange material, the correct work with ionexchangers were thoroughly discussed. Local specialists processed themselves (under my supervision) a second batch of "raw" immunoglobulins.

Detailed description of the ionexchange stages was written down by the author. The needed amounts of reagents and materials were calculated for a large scale production. The technological instructions were handed over to the Mongolian specialists.

CONCLUSIONS

The duties of an expert in blood derivatives production specified in the author's job description were fulfilled according to possibilities created in this first part of the mission:

1. The results achieved in the implementation of the project DP/MON/82 were consolidated by putting into action the Sartocon ultrafiltration module for diafiltration and concentraton of albumin solutions and by successfuly freeze-drying of two batches of immunoglobulins.

2. A programme for the thecretical and practical training of local personnel on the specific problems of the fractionation related to the production of immunoglobulin for intravenous use was prepared by the author. This programme was strictly followed.

3. New unknown in Mongolia technology was introduced for the production of "raw" immunoglobulin from placental material for intravenous use.

4. The second stage of the technological process, the ionexchange chromatography was carried out on a pilot scale and a new up-to-date process for production of high purity immunoglobulin for intravenous use was demonstrated.

RECOMMENDATIONS

In order to consolidate and fully utilize the achieved technological results it is recommended to:

1. Shorten my current mission and departure from Ulan Bator at the beginning of December and extend my second mission accordingly.

2. The second mission to be organized upon delivery of all equipment and after the accomplishment of training fellowships programmes.

3. Special attention to be paid to the future training of local personnel. It is recommended that the training of two fellows in new developments in the production of serum derivatives by means of fellowships be performed as soon as possible.

4. It is recommended too that a study tour of one local specialist in two countries on production of intravenous immunoglobulin be organized.

5. It is recommended that the goverment authority ensure regularly each year the necessary funds for import of reagents and materials needed for the production.

ACKNOWLEDGEMENTS

I would like to thank everyone associated with the planning of my work in Mongolia. My thanks to UNIDO for supporting this mission and to the Bulgarian Medical Academy for relieving me of my normal duties which permited me to carry it out.

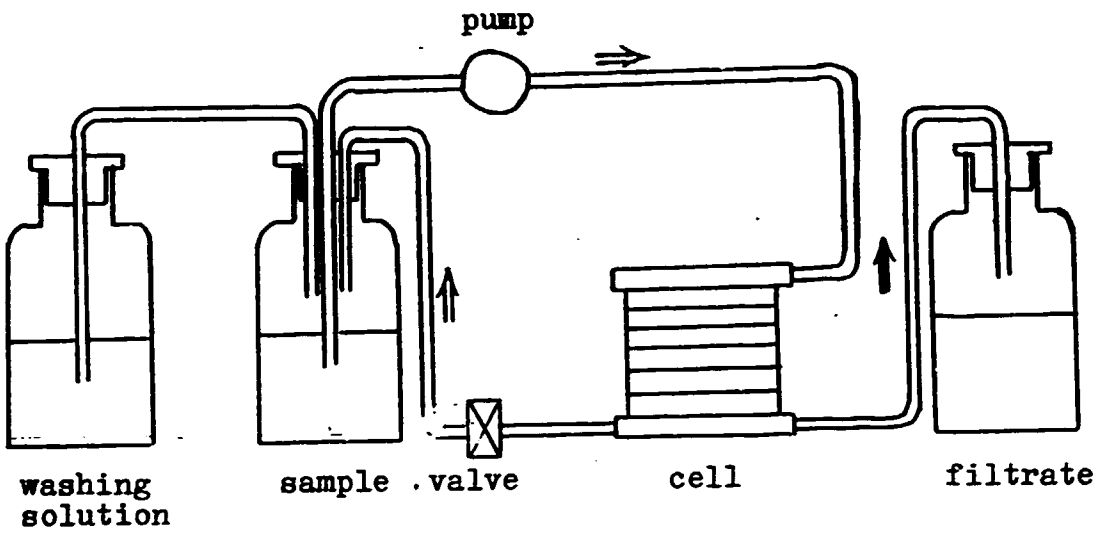
6. December 1988

UNIDO Expert in Blood Derivative Production:

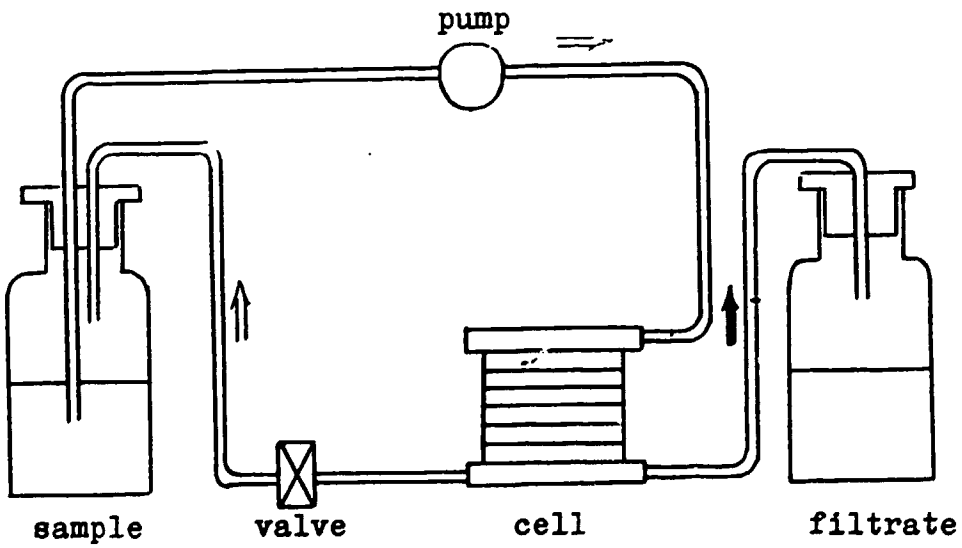
L. G. Bozadjiev

Annex 1

Operation in the constant volume washing mode



Operation in the recirculating mode



Annex 2

Fractionation scheme in the production of "raw" immunoglobulin for intravenous use from retroplacental serum

retroplacental serum
protein 7%
ethanol 8%
μ 0,14
pH 7,0-7,2
T -3 °C

supernatant
protein 5%
ethanol 25%
μ 0,09
pH 7,0-7,2
T -8 °C

precipitate
(discard)

supernatant
(for albumin)

precipitate
protein 1%
μ 0,003
pH 5,1
T 0 °C

supernatant
protein 0,6%
PEG 6000 3,5%
μ 0,003
pH 5,1
T 0 °C
ethanol 6%
T -2 °C

precipitate
(discard)

supernatant
protein 0,5%
ethanol 25%
μ 0,01
pH 8,0
T -8 °C

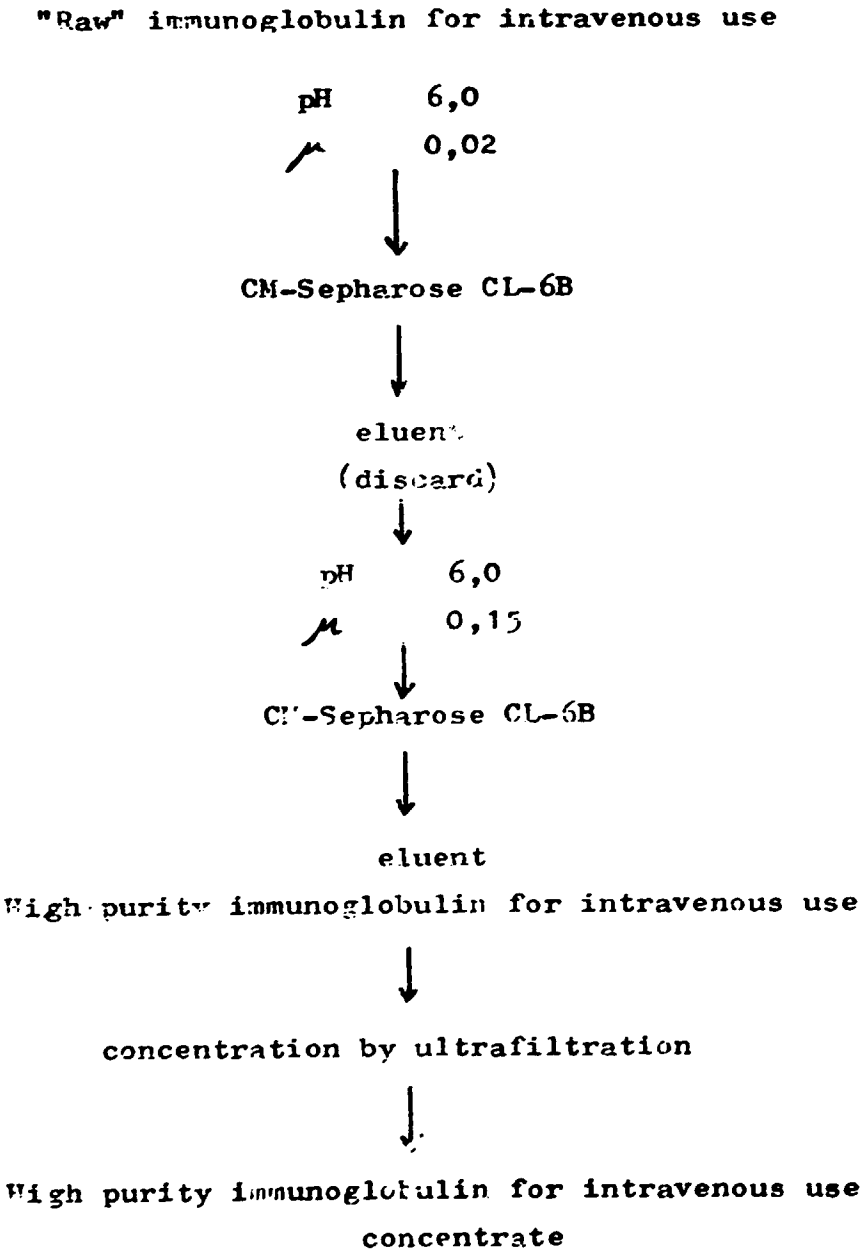
precipitate
(discard)

supernatant
(discard)

precipitate
"RAW" IMMUNOGLOBULIN FOR INTRAVENOUS USE

Annex 3

Ionexchange chromatography in the production of high purity immunoglobulin for intravenous use



Annex 4

PROGRAMME FOR THEORETICAL AND PRACTICAL TRAINING OF LOCAL PERSONNEL IN THE PRODUCTION OF BLOOD DERIVATIVES

(Course of lectures - November 1988)

1. The immunoglobulin preparation for intravenous use. The difference between this bioreparation and the normal human immunoglobulin.

2. The anticomplementary activity of normal human immunoglobulin preparations. The role of aggregation.

3. The specific problems of the fractionation related to the production of free aggregates and free of anticomplementary activity immunoglobulin preparations.

4. The method of fractionation with polyethylenglycol. The role of polyethylenglycol in the preservation of the native form of the immunoglobulin molecules.

5. Theoretical aspects of ionexchange chromatography.

6. The ionexchange chromatography and the ultrafiltration in the process of production of immunoglobulin for intravenous use. The role of the ingredients in the final formulation for the stability of the immunoglobulin molecules.

7. The therapeutic and prophylactic roles of immunoglobulin for intravenous use.

Practical training (November 1988)

1. Practical training on polyethylenglycol 6000 fractionation for the production of "raw" immunoglobulin preparation.

2. Practical training on the application of the fractionation equipment in the cold room (reactors, pumps, filters, colloid mill etc.) for the fractionation with polyethylenglycol.

3. Practical course on the technology for the production of "raw" immunoglobulin preparation for intravenous use in a production scale.

4. Practical training in diaphiltration and ultrafiltration.

5. Practical training on ionexchange chromatography on a pilot scale for the production of immunoglobulin for intravenous use with high purity and stability.

ABSTRACT

Project title: Programme "Biomed" - Introduction of new technology in blood fractionation

DP/MON/87/003

The objective of the present Follow-up Programme "Biomed" is the consolidation of the Blood Fractionation Unit and the introduction of a new technology to start the production of an immunoglobulin preparation for intravenous use.

At present the quality standards related to blood derivatives (immunoglobulin and albumin) produced in Mongolia meet the requirements of WHO.

The quality control procedures to be introduced for the characterization of the immunoglobulin preparation for intravenous use (produced from retroplacental serum) were discussed in detail with the local specialists. They were also trained in the specific control methods for this preparation. The first small experimental batches were tested and it could be forseen that soon they would be ready for clinical trials. A more complete characterization of the immunoglobulin preparations produced locally could be done (it was possible to determine immunoglobulin classes and IgG-subclasses). At present only anti-staphylococcal antibodies are determined in the immunoglobulin preparations. The antibody content of each lot of immunoglobulin must be studied and thus the preparations would be directed for the prophylaxis or treatment of definite diseases and be used more efficiently. The needed diagnostic preparations must be supplied.

In order to fulfil output 1 of the Project Document some expensive equipment is needed.

The Government should ensure the necessary continous service of the existing cooling installation and install as soon as possible new cooling machines.

INTRODUCTION

The Blood Fractionation Unit which now belongs to the Institute of Hygiene, Epidemiology and Microbiology is the only Unit which produces derivatives of human blood in Mongolia. The first UNIDO Programme "Biomed" 1985-1987 supported substantially the efforts of the Government to develop and modernize the Blood Fractionation Unit. The objective of the Follow-up Programme "Biomed" is the consolidation of this Unit and the introduction of a new technology (preparation of an immunoglobulin for intravenous use).

The author's mission in Ulan Bator started on 7 December 1988 and finished on 31 December 1988. The period from 31 December 1988 to 6 January 1989 was devoted to a travel for debriefing at UNIDO-Hq.

The author's activities were directed to the fulfilment of the duties of a quality control expert in blood fractionation and of a team leader.

The author's duties as a quality control expert were to:

1. Examine the state of control and registration of the parameters at the consequtive stages of the fractionation of placental materials.
2. Examine the state of quality control and standardization of albumin and immunoglobulin and compare to the requirements of WHO.
3. Train local personnel in control methods applied for the characterization of immunoglobulin for intravenous use.
4. Perform the control procedures in the production of the batch of immunoglobulin for intravenous use for clinical trials.
5. In cooperation with the blood fractionation expert and the maintenance engineer evaluate the work on the project.

The author's duties as a team leader were connected with general activities concerning the progress of the project.

ACTIVITIES CARRIED OUT

The state of control and registration of the parameters at the consequtive stages of the fractionation of retroplacental serum was examined. It was observed that records were made correctly on the performance of all steps in the manufacture and quality control. These records were detailed enough and made concurrently with the performance of each operation and test and signed by the person who did the work. The main information collected in the fractionation steps comprised variations in physical conditions (temperature,pH, ionic strength,timing,etc.) and reagents used. Unfortunately,because of the bad performance of the cooling installation sometimes the temperature conditions were not strictly followed. This might bring to great problems relating to the qualities of the products. At present the registration of the characteristics of the preparations showed that the qualities of immunoglobulin and albumin meet the requirements of WHO.

A programme and timetable for the author's activities in the theoretical and practical training of local personnel in control methods applied for the characterization of immunoglobulin for intravenous use was prepared and approved by the Director of the Institute. This programme included some points which were related to the author's general activities as a team leader.(Annex 1)

The quality control procedures to be recommended in the future production of the immunoglobulin preparation for intravenous use were discussed in detail with the National Project Coordinator and the specialists in laboratory control. The author suggested that the Blood Fractionation Unit and the control authorities characterize the immunoglobulin preparation for intravenous use by the following parameters (based on the recommendations of WHO):

1.Identity	of human origin
2.pH	6,9 ± 0,5
3.Total protein,%	6 ± 0,6
4.Immunoglobulin content,%	5 ± 0,5
5.Stabilizers	
a) albumin,%	1 ± 0,1
b) sodium chloride,%	0,9 ± 0,1
c) maltose,%	2 ± 0,2 or 2÷10

6.Molecular composition	
a) IgG-aggregates,%	less than 1
b) IgG-dimer and monomer,%	not less than 80
c) IgG-fragments,%	none
d) albumin	less than 20
7.Anticomplementary activity	5 mg or more of the preparation bind 2$CH_{50}^{'}$ units of complement of guinea pig
8.Blood group substances A and B	not more than 2 µg/ml
9.Proteolytic enzymes content	not more than 0,1 CTA units/ml
10.Polyethyleneglycol 6000 content	not detected
11.Stability	when stored at 37°C for 14 days the anticomplementary activity should be in the stated limit
12.Sterility	sterile
13.Abnormal toxicity	none
14.Venous tolerance	well tolerated
15.Pyrogenicity	pyrogen free
16.Hypotensive action	none
17.Activity	the antibodies in the final preparation to be concentrated at least 3 times or more compared to the starting material
18.HBsAg-content	none
19.HIV-antibodies content	none
20.Appearance	clear liquid

Most of the parameters (Nono 1,2,3,4,5ab,6,8,12,13,15,20) are routinely controlled in the production of normal immunoglobulin and are practiced well by the Mongolian specialists.

The author's efforts were directed to train local personnel and perform the specific control procedures to characterize the first experimental batches of immunoglobulin for intravenous use.

The methods of determination of venous tolerance (in guinea pigs),hypotensive action (in cats) were only discussed but not practiced.

The same was done about the method of determination of residual proteolytic activity. The training on these methods would be done in practice during the fellowships programmes.

Three small batches of immunoglobulin for intravenous use were analysed. Two of the batches (No 1 and No 2) were prepared under the guidance of the Blood Fractionation Expert. He also prepared the "raw" immunoglobulin from retroplacental serum (enriched by antistaphylococcal antibodies) by polyethyleneglycol/ethanol fractionation. The purification step of a portion of this antistaphylococcal immunoglobulin was performed successfully by the local specialists under the author'supervision (this was experimental batch No 3). The experimental batches were 400-500 ml each.

The routine tests (determination of protein content,hydrogen-ion concentration,determination of purity-electrophoresis and immunoelectrophoresis,sterility,freedom from undue toxicity,pyrogen test) were carried out by local personnel.

The author devoted special attention and time to the following tests: determination of anticomplementary activity,blood group substances,maltose and polyethyleneglycol, HBsAg-content.

A. Test for the determination of the anticomplementary activity of immuncglobulin preparations for intravenous use

The amount of protein which binds $2CH'_{50}$ units of complement is determined.

A short description of the method is as follows:

a) Reagents: versene-sodium buffer (μ=0,147), pH=7,4 ,containing Ca^{2+} and Mg^{2+} ions, lamb erythrocytes in buffer, hemolysine (titre 1:2400) and complement (100 units/ml). (Reagents were available locally.)

b) Procedure - in the first stage a dose of $2CH'_{50}$ units of complement was reacted with immunoglobulin dilutions (containing 20,10,5, 1 and 0,5 mg protein/ml); in the second stage the non-bound complement was determined in the presence of sensitized erythrocytes.After the incubation with the sensitized erythrocytes the samples were cooled and centrifuged and the supernatants were read at 540 nm. The least amount of protein which bound 2 CH'_{50} was found by the inhibition of hemolysis.

The following results were registered for the three experimental

batches of immunoglobulin for intravenous use:

No 1 - 1 mg of protein bound $2CH'_{50}$

No 2 - more than 5 mg of protein bound $2CH'_{50}$

No 3 - more than 5 mg of protein bound $2CH'_{50}$

B) Determination of blood group substances in the preparations

The method of inhibition of the hemagglutination of A and B human erythrocytes with anti-A and anti-B sera by the blood derivatives was applied.

The following procedures were performed: a) 3% suspensions of human erythrocytes A and B in saline were prepared; b) the highest dilution of the antisera (supplied from Behringwerke by UNIDO) which gave 4+ agglutination was determined; c) serial dilutions of the samples of immunoglobulin for intravenous use were prepared. Albumin from retroplacental serum was used as a positive control. To the series of dilutions anti-A and anti-B sera were added. After 30 min a drop of the erythrocyte suspension was added to every dilution and the agglutinations were read after another 30 minutes.

It was found that the samples of the batches of immunoglobulin for intravenous use gave no inhibition of the agglutination, i.e. the blood group substances were totally removed in the purification steps. The albumin preparation inhibited the hemagglutination, i.e. it had some residual blood group substances.

C) The content of the stabilizer - maltose was determined by the following simple colour reaction : 5% phenol solution was added to the samples. Then concentrated H_2SO_4 was added and after mixing the samples were put in a water bath at 30^0C for 10 min. The colour was measured at 490 nm. In advance a standard line on the relationship concentration of maltose to E_{490} was drawn based on the results obtained with definite amounts of maltose; the E_{490} of the tested sample was plotted on the graph to find the unknown concentration.

It was found that the quantity of the stabilizer maltose in the immunoglobulin preparation for intravenous use (three experimental batches) was within the limits.

D) The polyethyleneglycol-6000 determination performed by the method below showed that this precipitating agent was removed during the ion-exchange chromatography stage.

The protein samples were treated with equal volumes of 10% trichloracetic acid; the precipitated protein was removed and the supernatant was reacted with I_2/KI reagent in the presence of $BaCl_2$. After 15 min E_{540nm} was measured. In advance a standard line was drawn based on the results obtained with known amounts of PEG.

E) HBsAg was determined with the ABBOTT ELISA test kit (provided for the project by UNIDO). The instructions of the producer of the test were followed and it was found that the pools of raw materials and the first fractions in the production of blood derivatives were contaminated with HBsAg. The reactions of the final preparations were normally negative. The experimental batches of immunoglobulin for intravenous use gave negative reaction also.

The small experimental batches of immunoglobulin for intravenous use No 2 and No 3 gave good results in all tests. Unfortunately during one of the manipulations of taking samples from No 1, the batch was bacterially contaminated which lead to pyrogenicity and unsatisfactory anticomplementary activity. Recommendations were given by the author how to solve the problem.

It was also found that the experimental batches of immunoglobulin had no aggregated molecules in them (gel-filtration method).

In order to characterize more completely the immunoglobulin preparations produced by the Blood Fractionation Unit the following two tests based on the method of radial immunodiffusion were performed: the concentrations of IgG, IgA and IgM were determined by means of Behringwerke NOR-Partigen Immunodiffusion Plates and the IgG subclasses were quantitated with the ICN-Immunobiologicals test-kit. Both kits were supplied by UNIDO. The instruction leaflets were strictly followed. Interesting information was obtained. It was determined that the immunoglobulin preparations contained mostly IgG (nothing new), normally IgA was in the limits 1-2 % and IgM - 0,2-1%. It was noted that a lot of normal immunoglobulin which was found to contain higher content of antistaphylococcal antibodies had higher IgA content - 3,6%. The

same was observed with the experimental batch of immunoglobulin for intravenous use (No 3) which was produced from retroplacental serum which had higher titre of antistaphylococcal antibodies. 4,1% IgA and 1,7% IgM were determined in it.

It is known that normally within the IgG class the order of concentrations of the four subclasses is $IgG_1 > IgG_2 > IgG_3 > IgG_4$. The studies of the immunoglobulin preparations (8 normal immunoglobulins and 3 immunoglobulins for intravenous use) showed that no IgG_4 could be detected in them, IgG_3 was found in the range: 1,1-2,7 mg/ml; most of the preparations had IgG_1 and IgG_2 in the same range of concentration - 40-60 mg/ml (normally IgG_1 is 3-5 times more than IgG_2 in adult human serum). It is difficult to comment this result at present but it is known that when IgG_2-concentration is reduced it is associated with recurrent infections of the individual. May be the higher IgG_2 in Mongolian immunoglobulin preparations mean that the adult population is highly resistant to infections. These studies should be continued to obtain enough information, which would be the basis for more accurate conclusions on the prophylactic effect of preparations produced locally.

The above studies are part of the research programme of the Blood Fractionation Unit which was prepared in 1987.

The author would like to point out that an improvement of laboratory skills of local personnel could be registered. Fortunately most of the previous trainees of the author still worked at the Unit while others had left looking for better employment conditions.

At the Blood Fractionation Unit they have the reagents to determine antistaphylococcal antibodies only. The antibody content of each lot of immunoglobulin must be thoroughly studied and thus the preparations could be directed for the prophylaxis or treatment of deffinite diseases and be used more efficiently. Government authorities should supply the needed reagents.

Beside the above training-in-service activity at the Blood Fractionation Unit the National Project Coordinator (Dr.Dandii) and the author organized a discussion on the qualities of immunoglobulin and albumin produced from placental sources and the urgent need to pro-

duce these preparations from extract of human placentae in order to give to the clinics more of these needed blood products. The processing of placentae was initiated by the first Programme "Biomed" in 1985-1987 but still there are obstacles to fractionate placental extract on a regular basis. These are: the equipment for this purpose is inadequate and lot of manual labour is needed (an expensive decanting centrifuge is necessary to fulfil output 1 of the Project - produce regularly blood derivatives from placental extract) and the high price of some of the reagents (e.g.ethanol is expensive in Mongolia but this reagent is needed in large amounts). The conclusion of the discussion was that all efforts should be made to ensure the production of blood derivatives from human placentae. The consumption of blood products is increasing in Mongolia and the processing of all placental sources would bring to self-sufficiency in this respect. The discussion was attended by Dr.Selenge of the state control laboratory, Dr.Basanjav and Dr.Demberen of the laboratory for control of biopreparations, Ms Sainchimeg-technologist and Ms Enkhtuia - accountant.

The author could visit the virology department - the laboratory of hepatitis and the laboratory of AIDS. Dr.Ganbaatar and Dr.Badamdorj were glad to show their equipment and describe their activities. Both laboratories were well equipped with the support of WHO.

A meeting of the author with the Director of the Institute (Dr.Kupul) was arranged. The discussion was a very fruitful one. The Director discussed in detail the activities on the project with the author and the National Project Coordinator. It was agreed that:

a) The Institute should make efforts to ensure the timely supply of the needed reagents for the fractionation of placental materials;

b)The Institute should order the needed machines to renew the cooling system of the Blood Fractionation Unit as soon as possible. Till the new machines are put into action (after 1-2 years) proper maintenance of the existing machines should be done. A qualified mechanic in cooling installations should be appointed;

c)The Institute should order the diagnostic preparations for the determination of antibodies in immunoglobulin preparations;

d)The laboratories of hepatitis and AIDS should collaborate with the Blood Fractionation Unit in the control of raw materials and the final preparations.

e)The possible measures should be taken to prevent the employees

from leaving the Blood Fractionation Unit (in the period February 1987 - December 1988 11 new employees were appointed to replace those who left). This fact created difficulty in the running of the Unit.

CONCLUSIONS

The results of the tests performed during the author's mission and the registration of the qualities of the preparations produced at the Blood Fractionation Unit showed that immunoglobulin and albumin meet the requirements of WHO.

The temperature conditions during the fractionation stages are not strictly followed because of the bad operation of the cooling system which is practically worn out. Because of this, in the nearest future problems related to the qualities of the products (contaminations with undesired fractions, instability, etc.) could be expected.

Local personnel was trained in some specific methods for the characterization of the new preparation - immunoglobulin for intravenous use. The small experimental batches of this preparation were analysed and it could be forseen that soon they would be ready for clinical trials.

A more complete characterization of the immunoglobulin preparations produced locally was started by the determination of the contents of immunoglobulin classes and IgG-subclasses in them. A research programme is followed.

At present only antistaphylococcal antibodies are determined.

Human placentae are still not used as a source for immunoglobulin and albumin preparations. In order to fulfil output 1 of the Project Document some expensive equipment is needed. The author was assured that the needed reagents for the fractionation of placental materials would be timely supplied by the Government. With the expansion of the production it seems that the store space is inadequate. Attention should be paid to this problem also.

RECOMMENDATIONS

Government should ensure the needed continuous service of the cooling installation and provide and install as soon as possible new cooling machines so that the temperature conditions of the fractionation be strictly followed.

The needed reagents for the production and control (also for the determinations of antibodies' content) at the Blood Fractionation Unit should be timely supplied.

One Mongolian specialist should be trained abroad (2-3 weeks) on the clinical application of immunoglobulin for intravenous use.

A close cooperation between the Blood Fractionation Unit and the laboratory of hepatitis and the laboratory of AIDS should be established in order to achieve the regular control of raw materials and blood derivatives to avoid transmitting of infection.

30 December 1988
Ulan Bator

Ilina Bineva

ANNEX 1

Programme and timetable of the activities of I.Bineva from 9 December'88 to 30 December '88

9 and 10 Dec. Discussions with the National Project Coordinator and examination of the state of laboratory control in the production of blood derivatives

12 Dec. a)Visit at the UNDP office

b)Discussions with the specialists in laboratory control at the Blood Fractionation Unit on the methods to be demonstrated and the preparations to be studied

13 Dec. Start of the experiment - determination of subclasses of IgG

14 Dec. a)Start of the experiment - determination of IgG,IgA,IgM

b)Determination of residual polyethyleneglycol in a preparation enriched by anti-staphylococcal antibodies,produced by the new technology

c)Supervision on the application of the method of ion-exchange chromatography on CM-Sepharose CL-6B for the final purification of staphylococcal immunoglobulin performed by local specialists (experimental batch No 3)

15 Dec. a)Visit at the virology department

b)Supervision on the preparation of the final solution of the experimental batch of staphylococcal immunoglobulin for i.v.use and control of the required parameters.

16 Dec. a)Supervision on the preparation of an immunoglobulin solution for lyophylization. Supervision on the first stage of lyophylization.

b)Analysis of the first experimental batch of immunoglobulin for intravenous use by gel-filtration (the analysis was done at the virology department)

17 Dec. a)Preparation of the needed reagents for the performance of the method of determination of anticomplementary activity

b)Measurements and calculations for the quantitation of immunoglobulin classes and IgG-subclasses

19 Dec. a)Meeting with the Director of the Institute of Hygiene, Epidemiology and Microbiology

b)Determination of the titre of complement and preparation of standardized lamb erythrocytes suspension for the determination of anticomplementary activity

c)Preparation of dilutions of the studied preparations

20 Dec. a)Determination of the anticomplementary activity of the experimental batches of immunoglobulin for intravenous use

b)Meeting with representatives of the control authorities responsible for the quality control of biopreparations in Mongolia.Discussions on the qualities of blood derivatives of placental origin.

21 Dec.	a)Supervision on the application of the method of lyophylization
	b)Determination of blood group substances
22 Dec.	Ultrafiltration of albumin and discussions on the principles of the method
23-24 Dec.	Preparation of the Annual Project Performance Evaluation Report for UNIDO/UNDP
26 Dec.	Determination of maltose
27-28 Dec.	Determination of HBsAg in raw materials,fractions and final preparations
29-30 Dec.	a)Visit at the UNDP office
	b)Preparation of the author's technical report

Note:

The arrangement of the programme may seem somewhat illogical but the timetable was made so that the author could participate at as many training activities as possible.

ABSTRACT

Programme "Biomed": Introduction of a new technology in blood fractionation

Project number:DP/MON/87/003/11-03

The state of the equipment supplied by UNIDO for the implementation of the Programme "Biomed" (DP/MON/82/004) was examined and it was found that most of it was in a good state.

It was possible to repair the freeze-dryer and thus it is not necessary to call a specialist from the firm-supplier as intended.

Attention was paid to training local personnel on maintenance and repair of all equipment belonging to the Blood fractionation Unit.

The work done by this Unit is of great importance for the Health-care in Mongolia,the training of the technical staff is rather specific and that is why it is recommended that Government Authorities do their best to keep the employees working there.

The cooling system of the cold room should be replaced by a new one as scon as possible. Three new aggregates (Freon-12 type) with water cooling and capacity of 10,000 kcal are needed.

INTRODUCTION

The background of this mission was as follows. The first steps of the Blood Fractionation in Mongolia were made aboul 15 years ago. Till 1986 the equipment used was old fashioned,worn-out and of low capacity. In 1986 the UNIDO Programme "Biomed" (DP/MON/82/004) supported substantially the efforts of the Government to develop and modernize the Blood Fractionation Unit. Modern equipment was supplied by UNIDO. Thus facilities were created to carry out large scale fractionation of placental materials.

The present follow up programme "Biomed" (DP/MON/87/003/ aims at the consolidation of the achieved results, the initiation of new developments and the provision of further support and supervision.

My duties as given in my job description for a split mission of 1,7 month (for 1988 and 1989) were to:

1.Examine the state of the equipment supplied by UNIDO for the implementation of the Programme "Biomed" (DP/MON/82/004) and report my findings

2.Install and put into action the equipment for the removal of placental tissue from placental extract

3.Install and put into action the equipment for large scale chromatography

4.Train local personnel on exploitation and maintenance of all equipment supplied by UNIDO for processing of placental materials to produce albumin,normal immunoglobulin and immunoglobulin for intravenous use

5.Make recommendations on future maintenance and prepare list of spare parts needed for repair.

6.In cooperation with experts in blood fractionation and quality control evaluate the work on the project

7.Prepare a report setting out my findings and give recommendations on further action which might be taken.

ACTIVITIES CARRIED OUT

I worked in close cooperation with the UNIDO Blood Fractionation expert (Dr.Bozadjiev) and the National Project Coordinator (Dr.Dandii). I was surprized that two (out of four) local specialists whom I trained in 1986-1987 to work with the new equipment had left the Blood Fractionation Unit.New employees had been appointed.

I examined the state of the equipment supplied by UNIDO for the implementation of Programme Biomed (DP/MON/82/004) and found that:

a) The following equipment was in good state:

-Pressure vessel GB 320
-Transport container NB 200 (two of them)
-Two multisheet filterholders Pilot A 20Z
-Two single sheet filterholders DGF 30A

-Two pressure filter holders,Dia 293mm
-Two pressure pumps,membrane type
-Two pressure tanks,20 l
-Four peristaltic pumps
-Modular design ultrafiltration apparatus
-Mohno pump Netzsch NL 15A
-Laminar flow hood ASW-up 1200
-Homogenizer
-Two ph-meters
-Automatic ampoule filling and sealing machine
-Centifuge CEPA TZ-3
-Chest freezers (most of them were distributed to hospitals ; there are no problems with thesetill present)

b) The following equipment needed repair:

-The rotary pump Rubicon 0 (Seitz)
-freeze-drier USIFROID Type SMH 50
-the optical unit of the set of tools for automatic gel-chromatography (Pharmacia)

The repair of the pump Rubicon 0 was the easiest task I had.

Then I paid a lot of attention and time to the freeze-dryer USIFROID. Some spares had been timely supplied and this helped in my work. The compressor and the air injection system of the lyophilizer were repaired by me, the machine was put into action and then the lyophilization of two batches of immunoglobulin was performed in my presence.

The optical unit of the chromatographic system had one of the electronic cards burned, it was inexpertly touched by two local specialists and despite the efforts I made this unit has to be repaired by the firm supplier (Pharmacia).

Out of the equipment needed for the implementation of the Follow-up Programme "Biomed" a cooling centrifuge "Cryofuge" 5000 was delivered. This centrifuge was put into action and it was successfully used in the preparation of two small experimental batches of immunoglobulin for intravenous use. The centrifuge has a rotor of large capacity (6 l) with 1-liter vessels for centrifugation only. It needs supplements for smaller vessels and tubes which would broaden the scope of utilization of this centrifuge.

Further on I checked all the other equipment of the Blood Fractionation Unit. I noticed that the cooling system for the cold room was practically out of order. This cooling system was constructed and put into action in 1973 or 1974 long before the implementation of the UNIDO programme and at present it is quite worn out. With some effort I could repair the relay, the thermostat and the pressostat of the machine which refrigerated the cooling agent but I think that this machine before long will be irreversibly out of action. The other machines of the cooling system were not in a better state but they were still acting. They need constant maintenance and repair and one could forsee that these machines shortly would be totally worn out.

I continued checking the equipment and found that one of the rotors of the supercentrifuges was partly damaged, I did my best to repair it,did some work with a lathe-machine,ground it also and left it in a good state.

I had to do some electrotechnical work also. One of the distillators had a control panel which was not working properly and I replaced it with a new one.

The Director of the Institute of Hygiene and the National Project Coordinator asked me to help with the installation of a reverse osmosis system (RO 50) for the deionozation of water bought by WHO from Purite Ltd.,England. Icould install this also.

During my mission I had 21 work days (I worked on Saturdays also, but 7 and 8 November were national holiday) I could fulfil the above activities and could pay some attention to training the two new employees (Mr.Jargal and Ms Dolma) in maintenance and repair of most of the equipment supplied by UNIDO till present. I worked also in close contact with Mr.Ganbold and Mr.Boldbatar (my trainees of my previous mission) with whom we discussed lot of details, I could answer most of their questions and carry out in-service training.

CONCLUSIONS

The following duties of a maintenance engineer in blood fractionation specified in the job description for a split mission were fulfilled:

The state of the equipment supplied by UNIDO for the implementation of the programme "Biomed" was examined and it was found that most of it was in a good state. The freeze-dryer was repaired and it is not necessary to call a specialist from "USIFROID" (the UNDP officers in Ulan Bator intended to do this). The optical unit of the chromatographic system had a deffective electronic card (no spares of this kind are available in Mongolia; besides,it is not possible to foresee a damage like this because the optical unit is not furnished with its electronic scheme by the supplier) which should be replaced by the firm.

The cooling centrifuge (delivered for the fulfillment of the Follow-up programme "Cryofuge-5000" was put into action.

A survey of all equipment of the Blood Fractionation Unit was made and it was found that the cooling system of the cold room was worn out.

Attention was paid to training local personnel on maintenance and repair of all equipment belonging to the Blood Fractionation Unit. Training was performed in-service.

RECOMMENDATIONS

1.Still the Blood Fractionation Unit is not fully ensured with technically qualified staff for maintenance and repair of the equipment. It was most unfortunate that two out of four my previous trainees had left the Unit. As the work done by this Unit is of great importance to health-care in Mongolia and the training of employees is rather specific I recommend that Government Authorities do their best to give some benefits to people working well in this Unit so they would not look for another jobs elsewhere.

2.The cooling system of the cold room should be replaced by a new one as soon as possible. The new system would need three machines water cooled and charged with "Freon-12". Their capacity should be 10,000 kilocalories.

1 December 1988

UNIDO Maintenance Engineer

I.I.Kjurkchiev