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ASSISTANCE TO THE EXPERIMENTAL CENTRE OF APPLIED ENZYMOLOGY AND MICROBICLOGY IN THE PRODUCTION OF STERILE ENZYME PRODUCTS (CHYMOTRYPSIN, TRYPSIN AND PANCYPSIN)

DP/MON/82/002

MONGOLIA

Technical report: Production of Sterile Enzyme Products by a Compact Filling Line *

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 Professor Oleg Scedrov,
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United Nations Industrial Development Organization
Vienna

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1 SUMMARY

The four-month mission of the Chief Technical Adviser, the Expert Technologist and the Quality Control Expert, was completed by the end of May 1987.

The mission's main task was the setting up the new line for sterile enzyme production in the Experimental Centre of Applied Enzymology and Microbiology in Ulan Bator, Mongolia. The line supplier's, "Rota" Company, engineer was late, but arrived during the experts stay in Ulan Bator. He has installed and adjusted the new line machines, and put them into operation. The experts' team was present during the first week of the trial production, too. The trial production has to be run during the following six weeks.

The well established production of Pancypsin, chymotrypsin and dry bile on the pilot plant scale in the Centre was enlarged, before the experts' mission, by the production on the laboratory scale of trypsin, pancreatin, pepsin, peptone, hyaluronidase, ribonuclease, deoxyribonuclease, cytochrome C, and some others. The existing technologies in the Centre were discussed, the advisory services rendered during the whole experts' mission, and the choice of new products was advised for future production.

The new quality control methods of Pancypsin, chymotrypsin, trypsin, pancreatin, pepsin, and of raw materials, as well as the regular microbiological checking of sterile final enzyme products, were introduced during the mission. The Centre specialists were trained in them, including the run of the daily quality control protocols. It was found that the quality of the Centre products, tested during the mission, meets the requirements of the world market.

The Project Second Phase products were recommended: Pancypsin, dry bile, chymotrypsin, trypsin, pancreatin, pepsin, medical and food grade, peptone, and for latter on blood hydrolysates and bile acids, all of them made preferentially in bulk. Only a smaller part of Pancypsin, chymotrypsin and trypsin can be finalized as sterile powder in vials.

The production capacities have to be set to meet the expected local consumption, as well as the real export possibilities.

2 INTRODUCTION

2.1 Project Background

The problem of developing a pharmaceutical industry and particul utilization of waste products from slaughterhouses, was discusse with the Mongolian authorities concerned, and with the members o the UNIDO Project Formulation Mission during their stay in the Mongolian People's Republic in April 1980.

The pharmaceutical industry in Mongolia is still in its initial stage of development and is mainly confined to the production of dosage forms. The Government has given top priority to the proje which aims to increase the production of drugs and certain natur preparations by utilizing animal waste products from slaughter-houses. These wastes form one of the natural resources which Mongolia abounds in. The Government has already undertaken certa measures to establish a local industry for utilization of these indigenous raw materials with the purpose of manufacturing pharma ceuticals and other bioactive products.

The Experimental Centre of Applied Enzymology and Microbiology ha been set up on the premises of the slaughterhouse in Ulan Bator. The Centre has already developed its own production technology fc several sterile enzyme products such as Pancypsin, chymotrypsin, as well as dry bile. Pancypsin is a completely new product. This an enzyme mixture from sheep and goat pancreases, and is patented in Mongolia. Technologies for production of trypsin, pancreatin, pepsin (from pig and bovine stomachs), peptone, serum gonadotropir and some other important bioactive substances are at different stage of development. The Centre possesses now some facilities fc production of bioactive substances on larger scale and a relative small quality control laboratory.

The next step is the establishment of a multipurpose pilot plant capable of manufacturing bioactive products according to the country's need and its export possibilities, as well as to elabor and develop technological processes with the aim of their further scaling up and transferring to industrial production level. The Government has requested UNDP/UNIDO cooperation and assistance in establishment of the said plant and putting it into operation. In

the first phase UNIDO has already provided preparatory assistance by sending a team of subcontractor's experts to carry out a techn-economical survey, Project DP/MON/80/004. The Feasibility Study "Establishment of the Pilot Plant for Processing of Biochemical Products" was done by subcontractor POLYTECHNA-SPOFA, Prague, in December 1981.

The present Project DP/MON/82/002, "Assistance to the Experimental Centre of Applied Enzymology and Microbiology in the Production o Sterile Enzyme Products (Chymotrypsin, Trypsin and Pancypsin)", was prepared on the basis of findings and recommendations submitt by that mission and it reflects the results of discussions with the Government authorities concerned. The First Phase of the Project will cover the establishment of modern facilities for the production of sterile enzyme products, of strengthening the quali control, and the production of gonadotropin in bulk. The producti programme which is being proposed for this stage is based on the experience and results already achieved at the Centre and it envisages the manufacture of pharmaceuticals and enzyme products, both for local consumption and for export.

In 1985 there was the first mission of the Chief Technical Advise of the Project which, according to the increasing production of Pancypsin, chymotrypsin and dry bile, and to expected production of serum gonadotropin, prepared realization of the Project. The main task of this mission consisted in selecting of suitable phar maceutical equipment for production of sterile enzyme in vials, selecting of equipment and apparatus for the quality control labo ratory, and rendering of advisory services to the development programme of enzyme and hormone production. Consequently a plan f fellowship and study tours was prepared. The selected equipment was ordered through UNIDO. The main part of the equipme the line for sterile vials, was purchased from the "Rota" Firm, West Germany. The line consisting of washing machine for vials, sterilizing tunnel, filling machine, closing machine and labellir machine, with all necessary spare parts, and with the production capacity of about 3 000 vials per hour, was purchased in 1986 and delivered to the premises of the Centre in Ulan Bator in January 1987.

2.2 Official Arrangements

The Chief Technical Adviser (CTA) and the two experts, the Expert Technologist and the Quality Control Expert, started on 26 January 1987 with their briefing in UNIDO Headquarters in Vienna. The field mission in Ulan Bator began on 30 January 1987. The relevant Job Descriptions are attached as Annexes 5.1.1, 5.1.2 and 5.1.3.

The two experts' mission was shortened to two months, according to the last budget revision by the end of 1986, because of lack of funds. After beginning of the mission, the National Counterpart asked the extention of the two experts stay to four months, as it was arranged originally, and this was accepted.

By the end of May 1987 the CTA and the two experts left Mongolia for debriefing in Vienna.

2.3 Objectives of the Work

2.3.1 New "Rota" Line

Evaluate the existing situation in preparatory work in premises of the Experimental Centre of Applied Enzymology and Microbiology for establishment of the new line.

Cooperate in installation and adjustment of the "Rota" line with engineer of the Firm.

Help the setting up of the line into motion, and the trial production.

Train the Centre employees in the operation of the new line in sterile enzyme production.

2.3.2 Experimental Centre of Applied Enzymology and Microbiology Products and Technologies

Rendering of advisory services and recommendations on introduction of bioactive substances from animal sources and improvement of the quality of the final products.

2.3.3 Quality Control Activities

Evaluation of the quality of the existing raw materials for enzyme production and suggestions of the quality control methods for these raw materials.

Evaluation of the existing specifications of the raw materials, semi-products and the final products on the basis of international standards.
Suggest how to sample the raw materials, semi-products and the final products.
Introduction of the new quality control methods of enzyme final products.
Checking the enzyme products stability.
Evaluation of the existing equipment and apparatus in the Quality Control Department of the Centre, and recommendation of the procurement of new one needed.

2.3.4 Draft Plan of the Project Second Phase Document

Elaboration of the draft plan for the Project Document of the Project Second Phase, DP/MON/86/001, "Pilot Plant Establishment for Processing of Enzyme Preparations".

The CTA and two experts' Mission Work Programmes are annexed in Russian and English (5.2.1., 5.2.2., 5.2.3)

3 GENERAL ACCOUNT

- The Counterpart Organization was the Experimental Centre of Applied Enzymology and Microbiology (ECAEM) in Ulan Bator, belonging to the Meat and Canned Meat Plant in Ulan Bator, under the Ministry of Light and Food Industries of the Mongolian People's Republic.

- The National Counterparts were:
Mr. Z. Lhundev, mechanical engineer, Chief Engineer of the Meat and Canned Meat Plant in Ulan Bator, and
Mr. J. Tserendendev, biochemist, Director of the Experimental Centre of Applied Enzymology and Microbiology.

- The group of three experts, the CTA, the Expert Technologist and the Quality Control Expert, worked in field as a good team. They solved together almost all tasks, discussed the problems arosen, and helped each other and mutualy. It was notable that the professional view-points and the proposed solutions of problems, during the mission, were mainly the same with all three of them. Very often the experts worked in groups of two, or all three together. The last was mainly in solving new "Rota" line tasks and problems.
All this represents one of the reasons for preparing only one joint report by all three experts.

- The Technical Reports of Mr. V. Vávra, Expert Technologist, (of 15 May 1987) and Mrs. M. Cobanović, Quality Control Expert, (of 15 June 1987) were mainly incorporated in this Mission Joint Report.

3.1 Comments on POLYTECHNA-SPOFA Feasibility Study

This Study was prepared on 1981, and it is well and reliably done. The problem is that six years have passed and the Study recommendations cannot be accepted without revisions. The Study can serve as a ground for the Project Second Phase, but with some changes in the production capacities and even in the choice of the products.

For example, the dry bile production capacity in ECAEM was higher last year than it is proposed in the Study for the future pilot plant; the capacity of peptone production mentioned in the Study is in question now, because of the troubles of selling it on the world market; after present expansion of the Pharmaceutical Factory Tableting Unit, its capacity is 14 times the last year's capacity, and there is really no reason any more to have a separate Tableting Unit in ECAEM, etc.

3.2 Work Performed Before Leaving for Mongolia

3.2.1 CTA's Visits to "Galenika" Works in Belgrade

The CTA visited the two groups of ECAEM specialists trained in "Galenika" Works in Belgrade during October 1985 (four of them) and in November and December 1986 (three of them). Visits were paid on 25 October 1985, 17 November and 4 December 1986. The training work plans were discussed and arranged with the "Galenika" management, and discussions were held with ECAEM specialists. Besides, the CTA visited "Galenika" on 28 August and 3 September 1986 and 15 January 1987 and discussed the expected mission problems with the Quality Control Expert and other "Galenika" specialists.

3.2.2. CTA in Zagreb Scientific and Technical Libraries

Before leaving for Mongolia, the CTA had spent a month searching in Zagreb scientific and technical libraries. Papers were collected, photocopied, and some other important papers and patents not found in Zagreb and Yugoslavia were ordered from abroad.

The subjects were: chymotrypsin, trypsin, pancreatin, pepsin, rennet, cheese manufacture, hyaluronidase, ribonuclease, ATP, blood hydrolysis, serum gonadotropin, mainly manufacture and quality control methods. All gathered papers and patents were taken to Ulan Bator and handed over to the Mongolian specialists.

3.3 Enterprises and Offices Visited in Ulan Bator [x]

3.3.1 Ministry of the Light and Food Industries of the Mongolian People's Republic

A visit was organized for the three experts to Mr. Gunsendorj (5.3.4), the Head of the Department of the Ministry of the Light and Food Industries of the Mongolian People's Republic in Ulan Bator, on 23 April 1987. The CTA and the two experts explained the aim of their mission, and an interesting and useful discussion followed after that.

3.3.2 Meat and Canned Meat Plant

A visit by the three experts was arranged to Mr. M. Shamjansuren, the Director General of the Meat and Canned Meat Plant in Ulan Bator, on 10 February 1987. A very useful discussion was held. The CTA gave a lecture on the slaughterhouse blood utilization to the Ulan Bator Meat and Canned Meat Plant and the Scientific-Experimental Centre of the Foodstuff Industry specialists, what had been arranged by Mr. Lhundev, Chief Engineer of the Meat and Canned Meat Plant, on 22 April 1987. The matter is reported detailed in the paragraph 3.5.5.2. The slaughterhouse blood processing is a prime need of the byproduct utilization in all slaughterhouses in the world.

3.3.3 Pharmaceutical Factory

Following the request of Mr. Litoukhin,(5.3.2), Resident Representative UNDP in Ulan Bator, the team of three experts, CTA, Expert Technologist and Quality Control Expert, visited the Pharmaceutical Factory in Ulan Bator on 27 February 1987 and, after seeing the production plant, had discussions with the Factory Manager Mr. P. Luvsan-Namjil (5.3.5) and the UNIDO Project DP/MON/84/001 CTA Mr. M. K. Polievktov (5.3.3). The second, more detailed, discussion with Mr. Polievktov was organized on 7 March 1987.
The Factory was founded in 1932, and in its present form has functioned from 1952. During 1986 the turnover amounted to 15 million Tughriks, and the profit reached 4 million Tughriks. Last year production was 40 million pills, 8 million ampoules, 200 tons of extracts, and 8 million bandages. The Factory has 127 different products. There is a rather poor quality control laboratory, and

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[x] The persons met during these visits are listed in Annex 5.3.

research laboratory. The UNIDO Project DP/MON/84/001, with UNDP contribution of approximately $ 300 000, deals with the arrangement of Tableting Unit. The new equipment was ordered for the higher production capacity, 75 000 pills per hour or 280 million pills per year, working in two shifts. The arrival of the new equipment is expected soon. It will have 14 times the capacity of the Tableting Unit.

Taking into consideration the new production possibility of the Factory it would be advisable that the final form of drug production in Ulan Bator be concentrated in one place, especially the tablets production. For example, in ECAFE they will need pills of pancreatin and pepsin in amount of approximately 3 to 10 million pills per year, which represents 1% to 3.5% of the Pharmaceutical Factory production capacity, using the new equipment of the Tableting Unit. Such organization would contribute to the strengthening of the over all pharmaceutical production in the country; would avoid duplication of capacities, procurement of equipment and would result in a more economical production. It is important to mention that the Pharmaceutical Factory has gained considerable experience in the final form of drugs production, which is one of the great advantages of the production in one place.

3.3.4 "Biomed", the Institute for Production of Human Blood Derivatives

According to the request of Mr. Litoukhin (5.3.2), the team of three experts visited "Biomed", the Institute for Production of Human Blood Derivatives, on 26 February 1987. After seeing the Institute, discussions were held with Mr. Dandii (5.3.6), medical doctor and the Director of the Institute. The Institute was assisted by the UNIDO Project DP/MON/82/004, with UNDP contribution amounting to $ 343 000. The Project has been recently completed. In addition to the expert services and training of personnel, the Institute acquired production and quality control equipment. A new "Rota" filling machine for ampoules was also provided. The capacity of the filling machine will not be fully utilized. In any case, dealing with such extremely delicate substances as are human blood derivatives, it is not advisable to

use it for production of other pharmaceuticals. It should be noted that this production is everywhere in the world separated from other drug production.

3.3.5 Institute of Experimental Biology of the Mongolian Academy of Science

The Institute of Experimental Biology of the Mongolian Academy of Science visited the three experts on 5 March 1987, as suggested by Mr. Litoukhin (5.3.2). The Director of the Institute, dr. C. Janchiv (5.3.7), showed the laboratories of the Institute and afterwards had a very useful discussions. The Institute is well equipped with Hungarian apparatus for genetic research. They perform primarily a fundamental research. Among others they are occupied with experiments for L-lysine production from yeasts by biotechnology, and the hepatitis B vaccine development by recombinant gene techniques. They have well educated and trained staff, mainly young people, for the research work mentioned. All of them are biologists. Considering the current achievements of science, it would be useful to employ some biochemists, too.
The CTA took part in the formulation of the new Project Document DP/MON/86/006, "Development of Biotechnology and Genetic Engineering", of the Institute, on 16 February, 9 and 10 April 1987, as requested by Mr. Litoukhin (5.3.2) and dr. B. Dashnyam (5.3.8), the Deputy Director of the Institute. The matter is reportes in Annex 5.4.
Following the request of dr. Dashnyam (5.3.8), CTA gave a lecture in English to the staff of the Institute on 15 May 1987. The title was "What is Biochemistry".(A copy of the lecture is annexed as 5.5.) An interesting discussion followed after the lecture.
The CTA suggested establishment of cooperation with ECAEM and dr. Dashnyam showed interest for such a cooperation.

3.3.6 Scientific-Experimental Centre of the Foodstuff Industry

A meeting of the CTA and dr. G. Gombo (5.3.9), the Director of the Scientific-Experimental Centre of the Foodstuff Industry in Ulan Bator, was arranged by Mr. Lhundev, the Chief Engineer of the Meat and Canned Meat Plant, only on 28 April 1987. The CTA suggested to establish a close cooperation of the Scientific--Experimental Centre of the Foodstuff Industry and ECAE in the pepsin utilization for cheese production, as well as in slaughterhouse blood processing. The subjects are reported detail in the paragraphs 3.5.5.1.1 and 3.5.5.2.

3.4 New "Rota" Line

3.4.1 Arrangement of Premises for the Sterile Enzymes Production before the "Rota" Line Installation

Starting their field mission, the experts found the new "Rota" line machines still in wooden crates kept on the ECAEM premises. It was decided not to open the crates before the "Rota" engineer's arrival.

The "Rota" engineer arrival was not well organized. Bigger part of experts' stay in the field, the exact date of his arrival was not known.

Because there was no technical documentation delivered with the "Rota" equipment, the drawings for future sterile rooms were not prepared properly, and the premises of ECAEM had not been arranged completely for the expected installation of the new line machinery. The experts' first main task was to prepare, in cooperation with the ECAEM specialists, the exact plans for the location of future production rooms and for all necessary preparatory work for the installation of the whole line. New drawings the rooms were prepared and all steps needed for the arrangement of the sterile rooms had been done, so that they should meet the rules for correct sterile production (Annex 5.6)

The already prepared rooms for the new "Rota" line still do not meet all the conditions of sterile production premises. There remained some tubings, central heating radiators, and there is still no air-conditioner for the sterile room, that would bring fresh sterile air into the room, improving the work conditions, and create a small overpressure inside the room to protect the sterile area against possible contamination from outside. In such a short time after experts' arrival it was not possible to arrange all the necessary work, and the remainder can be done after the installation of the new line, not jeopardizing the start of the trial production of the line (4.1.2.1, 4.1.2.2, 4.1.2.3 and 4.1.2.4)

The existing two freeze-drying machines were of the smaller production capacity than the "Rota" line. The "Rota" line capacity per shift was (3000 x 8 =) 24 000 vials, and the two "Leibold"

freeze-dryers capacity per day amounted to 13 200 vials, (2200 vials for 6 hours on each machine, 2200 x 2 x 3 = 13 200 vials per day). A new freeze-drying machine will be needed to balance the production capacity (4.2.1 and 4.2.3.3).

3.4.2 "Rota" Line Documentation

From the very beginning of their stay in field the experts, as well as the ECAEM Director and specialists, asked for any technical documentation, which was supposed to have been delivered with the "Rota" machines. Only a month and half latter, on 17 March 1987, there arrived the expected documentation for single machines. It was found that this documentation was not complete, it contained many errors, and changes were made in the sizes of machines to such an extend, that some reconstruction of already finished preparatory work on sterile production rooms, had to be made. A short report was prepared on the findings (Annex 5.7).
On the National Counterpart's request the Expert Technologist worked out on 22 April 1987 the "Short information on the function of Rota line equipment", using the available "Rota" documentation (Annex 5.8).

3.4.3 Sterility Problems Solution

3.4.3.1 In the New "Rota" Line Premises

During the whole missions the experts instructed the ECAEM specialists and workers, orally and in written, how to arrange the sterile rooms for the new "Rota" line, and how to keep sterile conditions in them. The written instructions are annexed (5.9 , 5.10, 5.11, 5.12)

3.4.3.2 Workers Gowns and Hygiene as well as the Workers Behaviour in the Sterile Premises

At the same time the experts instructed the ECAEM employees how to prepare the workers gowns for the sterile premises and how to secure the sterile conditions of the gowns, as well as how to keep the workers hygiene needed in the sterile conditions.
The workers behaviour in the sterile conditions of the new "Rota" line premises was covered by the instructions, too.
All Annexes quoted in the previous paragraph (3.4.3.1) relate also to this one.

3.4.4 Installation and Adjustment of the Line

According to the Mission Work Programme (5.2.1) the installation of the "Rota" line should have started on 15 February 1987, and the main work, securing good technology in preparation of sterile products, training of ECAEM workers to operate the line and run the trial production, all should have been completed by 15 April 1987.

The "Rota" Firm Engineer, Mr. K. Randjelovic (5.3.13), arrived later on and started work in ECAEM on 3 May 1987. From the very beginning of his stay he announced that he would spend in Ulan Bator no more than ten days. This was contrary to the experts and the counterparts expectations that he was to stay longer, during part of the trial production too, which would be understandable.

The "Rota" Engineer (5.3.13), in cooperation with the experts and andthe ECAEM specialists and workers, opened and controlled the delivered crates, and arranged for all necessary steps for the installation. Two machines, the sterilizing tunnel ST-1 and the washing machine Juffy I, were damaged, but not so much as to hinder the installation work. The accurate description of damaged parts is given in the Protocol on delivery of the "Rota" line, which is annexed (5.13).

Only crate No. 9, with labelling machine RE-50, contained some documentation. This means that there is still no correct documentation for the filling machine FLR-50/G and crimping machine FLR-50/B. According to the "Rota" Specialist (5.3.13), the documentation for the combined machine FLR-50/GB, which has been sent in March 1987, can be used because both machines are rather similar. However, the experts asked for sending the correct documentation for each machine.

The main installation problem, that the sizes of some machines were changed without sending any information on time, was solved with provisional connection arrangements. The "Rota" Engineer (5.3.13) promised to send all the extension parts and tubings after his return to the "Rota" Firm. These parts includes one transparent shield over extended chute between washing machine and sterilizing tunnel, and one stainless steel tubing and three teflon tubes for connection of washing machine with pump-over agregate. All this was confirmed in written in the Protocol

on the installation of the "Rota" line (Annex 5.14).
The "Rota" Engineer (5.3.13) informed then about the need of compressed air for all the machines of the line, which was news, because in the documentation from March 1987 only two machines were mentioned that would need a compressed air supply. In ECAEM they had one compressor of insufficient capacity and a rather old one. It was not possible to supply the compressed air for all machines at once. First the washing machine was filled with compressed air, after that the sterilizing tunnel, and so step by step. The labelling machine was the last one, and thus it functioned
It was arranged with the ECAEM Director to purchase and install a new compressor of a bigger capacity as soon as possible (4.1.2.2).
During installation of the line, which was done in good cooperation with maintenance workers, the "Rota" Engineer (5.3.13) trained the future maintenance workers in all the necessary knowledge as how to keep the machines in good repair and how to do all common repairs on them.
During finalization of installation of single machines the future workers on the equipment were trained in the correct operation and run of all machines. All questions by employees, were immediately explained. Nevertheless, the experts asked Mr. Randjelovic (5.3.13) to prepare short working instructions in written for every machine, i.e. when to press each button during the operation of the machine, how to start and stop the machine. There was a complete lack of such necessary short instructions in all "Rota" documentation. Mr. Randjelovic (5.3.13) promised to do it, but he did not succeed, because of his too short stay in Ulan Bator. So, he promised to send it after return to the "Rota" Firm as soon as possible, and that was confirmed in written in the Protocol, too (5.14).
After finishing the installation of the whole line, there remained a wall to be built between the sterilizing tunnel and the sterile room with filling machine, and to finish tubing for the supply of compressed air, which was done during the week-end.

3.4.5 Putting the "Rota" Line into Operation

It was decided, on the request of the Counterpart, that the putting the line into operation be at the same time the first trial production. It was on 11 May 1987, the last day of Mr. Randjelovic (5.3.13) work in Ulan Bator. All was arranged, the sterile room, the workers gowns, the Pancypsin solution prepared. One of the most responsible duty befell the ECAEM microbiologist. The "Rota" Engineer (5.3.13) was in the sterile room with the filling machine. A batch of approximately 2000 filled vials of 25 mg of Pancypsin was processed. So, the first trial production was run under the supervision of the "Rota" Engineer and it was mainly successful. Only the volume of filled solution in vials was not properly adjusted, and instead of 25 mg of product, 30 mg was filled. (On the next day this was corrected.)

A new problem arose during the putting of the line into operation. The printing head of the labelling machine was not printing properly because of different heights of the changeable type. It was decided that Mr. Randjelovic (5.3.13) take the wrong type to the "Rota" Firm for correction, and after repair send them back to ECAEM as soon as possible. This was confirmed in the Protocol, too (Annex 5.14).

After finishing the putting the line into operation and the first trial production, the necessary documents were prepared:

- "Protocol on Delivery of Rota Filling Line Equipment"(Annex 5.13), and
- "Protocol on the Installation of Rota Automatic Filling Line for Production of Sterile Products in Vials" (Annex 5.14).

Both documents were discussed and approved by the Resident Representative (5.3.2) at UNDP Office, and were signed by the Counterpart, the CTA and Mr. Randjelovic (5.3.13).

The whole "Rota" line was taken over by the Mongolian side in a good production state.

According to the Protocol on the installation of the "Rota" line (Annex 5.14), the guarantee period will be half a year, starting on May 1987. Later on (29 May 1987),during the debriefing in UNIDO Headquarters in Vienna,the CTA discussed the problem with dr. Csizér, the Substantive Officer, and Mrs. Gauss, the Supplying Officer. The steps will be taken that the "Rota" Firm extend the guarantee period to one year, what is usual for such a costly equipment.

3.4.6 Run of the "Rota" Line Trial Production

According to the Mission Work Programme (Annex 5.2.1) the trial production of the "Rota" line has to run for six weeks. Because of the delay of the "Rota" Engineer arrival, the experts were present only during the first seven days run of the line, from 11 May to 19 May 1987, the last day of their stay in field. All the remaining trial production run had to be done by the ECAEM specialists, up to 20 June 1987.

3.4.6.1 Trial Production during the Experts' Stay in Ulan Bator

The experts were deeply involved in the trial production process during the first seven days run of the line.
Before the trial production started, they arranged with Mrs. T. Adjasuren, the microbiologist, and Mr. K. Kabden (5.3.10), the pharmacologist, the programme of a very detailed microbiological and pharmacological continuous control, that has to be carried out during the "Rota" line entire trial production period.
It was agreed on the every-day production capacity, the processing of 65 g of Pancypsin. The volume of the filled Pancypsin solution in a vial was set to the correct 25 mg per vial.
The "Rota" line was in a good condition and worked satisfactorily.

3.4.6.1.1 Microbiological Control during the Trial Production

The CTA and the Quality Control Expert prepared, on 29 April 1987, with the ECAEM microbiologist, the daily Work Programme of microbiological control of the complete trial production process of the "Rota" line, including the sterility control of air in the premises, of walls, workers hands and gowns, distilled water, empty vials and stoppers, as well as enzyme product in different production steps including the final product (Annex 5.1.5). They thoroughly discussed the everyday examination results and suggested to the microbiologist the future work.
The sterile rooms were sterilized by ultraviolet lamps and the floors, walls, and ceilings washed with a chloramine solution daily.

It was a problem of sterilization of gowns and gear because of the single autoclave, of a too small capacity, that was available in ECAEM. The second one would be needed badly to ease the work of the microbiologist (4.1.2.4).
The microbiological control was done in a correct way.
The figures of the microbe number were checked by the experts every first seven days run of the "Rota" line. Sterilization tunnel worked correctly, and all other examinations were satisfactory. Only the first and third days runs, on 11 and 13 May 1987, the final product in vials were contaminated. That is understandable, because of the beginning of the work of the completely new equipment.

3.4.6.1.2 CTA Comments after Stay in Sterile Rooms of the "Rota" Line

The fourth day of the "Rota" line trial production, on 14 May 1987, the CTA spent about two hours in the sterile rooms, properly dressed. His observations, remarks and suggestions are annexed (5.16). The main problem in the sterile rooms was bad air without ventilation, and the chlorine smell. The lack of an air-conditioner is a serious problem, which has to be solved shortly (4.1.2.1). Other comments deal with some improvement in the sterile work techniques and on reduction of the furniture (stools, table) in the sterile rooms.
On the same day a meeting was arranged after that with the ECAEM Director, Chief Engineer, microbiologist and workers in the sterile rooms of the "Rota" line. The CTA listed all his observations and suggestions and discussed the matter with the participants very thoroughly.

3.4.6.1.3 Pharmacological Control during the Trial Production

The CTA and the Quality Control Expert prepared on 6 May 1987, with the ECAEM pharmacolcgist, the daily Work Programme of pharmacological control of the trial production process of the "Rota" line. The pyrogen and toxicity examinations of distilled water and the final product in vials, were included (Annex 5.17). Every examination quoted has to take ten days, and during the experts'

stay in the field no result was available. The pharmacological control was (and has to be) done every day during the "Rota" line trial production.

3.4.6.2 Run of the "Rota" Line Trial Production in the Experts' Absence

The matter will be considered in the paragraph 4.1.1.

3.5 Products of ECAEM

3.5.1 Experts' Advisory Services to ECAEM

During their stay in the field the experts were acquainted with the existing production and the production possibilities of ECAEM. The CTA rendered the advisory services to the Counterpart and to the ECAEM specialists in order to improve and develop their bio-active products, to introduce new production procedures, and some new quality control methods. He provided them with certain technical papers and patents, as quoted in the paragraph 3.2.2.
During the whole period of stay in the field the Expert Technologist cooperated with technicians of ECAEM, discussing with them all the technological problems they were interested in, and giving them advisory services on the already developing technologies or on possible new, interesting biochemical drugs for production in the future.
All production technologies and production schemes annexed here, were prepared by the Expert Technologist.

3.5.2 ECAEM Production Unit on the Pilot Plant Scale

In the ECAEM Biochemical Production Unit they are running one film evaporator, Anhydro, Denmark, with the capacity of approximately 50 lit of evaporated water per hour, and one spray-dryer, Anhydro, Denmark, with the capacity of approximately 3 kg of dry product per hour. Both machines are utilized now for dry bile production in two shifts, only in summer when the slaughterhouse is operating. Besides, there is one separator, Westfalia KA-30-06-076, West Germany, which is not fully utilized now, and four stainless steel vessels for extraction, as well as several stainless steel sieves. There is no heating in winter, and the equipment cannot be run during the whole year.

3.5.3 ECAEM Products Well Established and Run

There are some well established and run products in ECAEM, such as Pancypsin, dry bile, and chymotrypsin and trypsin.

3.5.3.1 Pancypsin Production

The Pancypsin production is highly developed in ECAEM. A group of the ECAEM specislists obtained the patent document for the Pancypsin production. Pancypsin is mainly a mixture of proteolytic enzymes, produced from sheep and goat pancreases. ECAEM is producing 35 kg of Pancypsin per year, partially in form of 25 mg sterile freeze-dried powder in vials, partially in form of 5 g and 100 g packages of substance in bottles. During the last days of the experts stay in Ulan Bator, it happened so that ECAEM sold to the Mongolian Ministry of Agriculture one million vials of Pancypsin for 2 million Tughriks. This amounts to 25 kg of Pancypsin substance. According to the Expert Technologist evaluation of the existing situation on the world market, the production should be increased, because Pancypsin is of a quality that meets or even exceeds the one requested by the well known world pharmacopoeias. The product could be easily salable in bulk, locally and for export, for possible production of antiinflammatory pharmaceuticals, and partially salable in form of sterile vials. In ECAEM they utilizes only sheep and goat pancreases from Ulan Bator slaughterhouse, the amount of which rather limits a possible increase of production. The experts suggested the use of cattle pancreas as well, to boost the Pancypsin production.
The Pancypsin production technology is annexed (5.18.1), as well as the production scheme (5.18.2).

3.5.3.2 Dry Bile Production

The dry bile production is well established in ECAEM. In 1986 produced was 4.5 tons of dry bile and sold abroad. The possible increase of quality of the product was discussed with technicians of ECAEM. An increase of the production capacity was suggested by the experts, too.
The production technology (5.19.1) and the production scheme (5.19.2) are annexed.

3.5.3.3 Chymotrypsin and Trypsin Production

In ECAEM they produce both enzymes from cattle pancreas, and they use the yak pancreas for the production, too. They have the technology elaborated and introduced, but there still remain some problems of adapting the technology to get good yields of both enzymes. The developing technology is oriented to the production of ribonuclease and deoxyribonuclease beside these two enzymes(3.4.5.6) and then it might be economically profitable. Chymotrypsin and trypsin are produced mainly in bulk, but a certain amount can be produced as 10 mg sterile powder in vials, too. The Expert Technologist discussed thoroughly the whole matter with the ECAEM specialists. According to him, trypsin in bulk is selling well on the world market, but with chymotrypsin some problems might occur.As Pancypsin, chymotrypsin and trypsin are produced on the same equipment, it is quite easy to change the production according to the existing situation.
The chymotrypsin and trypsin production technology(5.20.1) and the production scheme (5.20.2) are annexed.

3.5.4 ECAEM Production on the Laboratory Scale

There are in ECAEM several products with the production technology laborated on the laboratory scale, such as pancreatin,peptone, medical grade pepsin, hyaluronidase, ribonuclease, deoxyribonuclease, serum gonadotropin, cytochrome C, etc.

3.5.4.1 Pancreatin Production

The production technology is well developed and tested on the laboratory scale. The product obtained is of a very good activity. Nevertheless, some technology improvements were suggested by the Quality Control Expert, on the basis of the assay performed.

A good experience has to be gathered in ECAEM on a larger scale production immediately.
Pancreatin represents a very important substance for pharmeceuticals prduction for healing of digestive difficulties. It is a mixture of digestive enzymes, amylase, protease and lipase.

Pancreatin is finalized in form of coated pills and as the expert. Technologist says, it is easily salable on the world market. The production technology (5.21.1) and the production scheme (5.21.2) are annexed.

3.5.4.2 Medical Grade Pepsin Production

The medical pepsin is produced from pig stomach mucose. ECAEM has technology developed and tested on the laboratory scale, and they need experience in production on a larger scale. Pepsin of good quality can be expected. The possibility of selling it on the world market are relatively good, in the Expert Technologist's opinion. His suggestions were passed over on possible improvement of stability of the product.
The production technology (5.22.1) and the production scheme (5.22.2) are annexed.

3.5.4.3 Peptone Production

In ECAEM they have a certain experience in laboratory scale production of peptone using paunch of cattle as raw material. They need experience in a larger scale production. From the used raw material, and utilizing the rather simple technology, it is possible to obtain product of mediocre quality. According to the Expert Technologist, some problems may occur in selling it on the world market.
The production technology (5.23.1) and the production scheme (5.23.2) are annexed.

3.5.4.4 Serum Gonadotropin Production

According to the information gathered, in ECAEM they have the serum gonadotropin production elaborated, and it is rather easy. The main problem is the raw material, the pregnant mare blood collection, and this problem has to be solved (4.2.2.1). Serum gonadotropin should be produced in the final drug form as freeze--dried powder in vials for veterinary usage. The CTA and the Expert Technologist discussed the whole matter with the ECAEM specialists.

3.5.4.5 Hyaluronidase Production

The production technology from bull testicles as raw material was elaborated in ECAEM on the laboratory scale. The CTA discussed the matter with the ECAEM specialists and gave them technical papers, especially on a hyaluronidase new quality control method.

3.5.4.6 Ribonuclease and Deoxyribonuclease Production

They elaborated the production technology on laboratory scale, according to the information gathered in ECAEM. Pancreas serves as a raw material, and trypsin and chymotrypsin are produced beside ribonuclease and deoxyribonuclease from the same glands (3.5.3.3) The experts discussed the matter with the ECAEM specialists. They will collect more experience in this production.

3.5.4.7 Cytochrome C Production

In ECAEM they produced a completely new product from horse and cattle heart, the cytochrome C. They will gather more experience in the production technology as well in quality control procedures. In any case cytochrome C may be an interesting product for sale on the world market. The experts discussed thoroughly the matter with the ECAEM Director and specialists.

3.5.5 ECAEM New Products

In ECAEM they have very good ideas concerning new products. From among others, cited here will be the most advanced ones, food grade pepsin, blood processing and the bile acids.

3.5.5.1 Food Grade Pepsin Production

The raw material for the food grade pepsin production is cattle stomach mucose, and the production procedure is rather similar to the pepsin processing from pig stomachs.
Several photocopies of the papers and patents on pepsin production from bovine stomach mucose and its quality control, were handed over by the CTA during February 1987 to dr. J. Alimaa, the Chief Engineer of ECAEM.
Food grade pepsin is mainly used as milk clotting enzyme in cheese manufacture.

3.5.5.1.1 Cheese Processing by Pepsin

A copy of Mr. V. A. Tumanyan's UNIDO Report "Assistance to the Dairy and Cheese Production by Improvement of the Raw Milk Tests and the Quality Control", SI/MON/85/802, January 1987, pp. 73, was given by Mr. Litoukhin (5.3.2) to the CTA on 27 February 198 The CTA immediately have studied the Report. His comments are annexed (5.24).

A meeting of the CTA and dr. G. Gombo (5.3.9) was held on 28 Apri 1987. During the very useful discussions, some papers on cheese making by pepsin were given to dr. Gombo, as well as two samples of pepsin from ECAEM. Dr. Gombo (5.3.9), who is a dairy specialis was very interested in cheese making using bovine pepsin, especia in sheep cheese manufacture. He has some experience in this field and he promised to perform test production in Ulan Bator Dairy Plant. Before Mr. Tumanyan's activity in Ulan Bator, dr. Gombo had performed Switzerland-type cheese trials, sometimes in 1970. The problem was a very small local consumption of such cheese. He will try now to sell cheese abroad. The sheep milk collection method has to be solved in Mongolia. According to dr. Gombo (5.3.9) estimation, approximately 50 kg of bovine pepsin will be needed pe year, with milk clotting activity of 1 : 100 000, for clotting of about 2 000 tons of milk, and production of about 720 tons of cheese. After a few years, he expects an increasing of cheese production and of pepsin consumption.

Dr. Gombo (5.3.9) asked the CTA to translate for him a very interesting Greek paper on sheep cheese production by pepsin, from French to Russian, what was done and the translation given to him on 6 May 1987.

The CTA proposed to establish a close cooperation of the Scientific-Experimental Centre of the Foodstuff Industry and ECAEM in the pepsin utilization.

3.5.5.2 Slaughterhouse Blood Processing

The first and foremost problem of animal byproducts utilization is the slaughterhouse blood processing. The main reason for, and the advantages of, blood processing are: first, its products are very valuable and second, it is a way of reducing environmental pollution.

The CTA suggested the blood hydrolysate and the plasma production to ECAEM, to the Ulan Bator Meat and Canned Meat Plant, and to the Scientific-Experimental Centre of the Foodstuff Industry. Mr. Lhundev, the Ulan Bator Meat and Canned Meat Plant Chief Engineer, arranged on 22 April 1987 a meeting of the Ulan Bator Meat and Canned Meat Plant and the Scientific-Experimental Centre of the Foodstuff Industry specialists, when the CTA gave the lecture in Russian on the slaughterhouse blood utilization. (The English translation of the lecture is annexed 5.25). A useful discussion was held after the lecture with many questions of the participants. Some papers and a West German patent about it, together with a drawing of the slaughterhouse blood processing, were given by the CTA to the participants of the lecture, as well as to Mr. Lhundev and to dr. Alimaa, the ECAEM Chief Engineer. The blood cells hydrolysis, and plasma as well as albumin processing, represent one of the best ways of blood utilization on industrial level.

During the meeting of the CTA and dr. Gombo (5.3.9) on 28 April 1987, the slaughterhouse blood processing was discussed as well. Some papers on the matter were given to dr. Gombo, too. The Scientific-Exerimental Centre of the Foodstuff Industry consists of several departments. One of them deals with meat products, and the specialists were interested in the slaughterhouse blood processing, and discussed the matter with the CTA.

A close cooperation was suggested by the CTA to ECAEM and to the Scientific-Experimental Centre of the Foodstuff Industry also in the blood processing.

3.5.5.3 Bile Acid Production

The dry bile is a very good salable ECAEM product on the world market (3.5.3.2). Purification of the dry bile will lead to bile acids, products more valuable than dry bile. This possibility was seriously discussed by the experts with the ECAEM Director and specialists.

3.6 Quality Control Activities

3.6.1 Existing Conditions of the Quality Control Activities in ECAEM

Running quality control in ECAEM was practised, before the experts' mission, for only final products such as Pancypsin, chymotrypsin, trypsin and dry bile. Quality control methods used in ECAEM for these products were a bit outdated.

Chemical and microbiological analyses in ECAEM were carried out in two separate laboratories. The space they occupied was very small for the performance of various operations and for the preparation of reagents.

The chemical quality control laboratory was of less than ten square meters in size and not provided with the suitable equipment for accurate and precise testings, with an adequate and sufficient amount of glassware and as well with proper purity grades of reagents utilized for enzyme assays. Washing and drying of laboratory glassware took place in a too small access corridor. The balance unit was located rather far from the quality control laboratory. The employees had to pass through the corridors and other premises in order to determine sample weight, with increasing possible risks of error through air and mechanical contamination. The laboratory was also inadequate supplied with manuals and pharmacopoeias needed for carrying out the quality control methods.

The microbiological laboratory was rather well equipped for the bacterial growth and activity, as well as for sterility examinations, but the work area was also too small.

The Quality Control Expert's "Evaluation of the Quality and Specification of the Existing Raw Materials, Semi-Products and Final Products at ECAEM" of 23 March 1987, is annexed (5. 26).

The corresponding recommendations can be found in the paragraphs 4.1.4.2 and 4.1.4.2.1 to 4.1.4.2.7.

3.6.2 New Quality Control Methods in ECAEM for the Final Products

In order to improve and standardize the quality of the enzyme and bioactive products, new methods for the ECAEM final products were introduced by the Quality Control Expert.

3.6.2.1 Pancypsin in Bulk and Final Drug Form

Following new quality control methods for Pancypsin were introduced in ECAEM:

- Determination of the total proteolytic activity by the Armour method; the enzyme activity is expressed in Armour units per mg.
- Determination of the relation of trypsin and chymotrypsin in the enzyme mixture; the activities are expressed in the United States Pharmacopoeia (USP) XXI units per mg of Pancypsin.

Total proteolytic activity according to the local requirements was previously determined in ECAEM by using older testing methods after Anson and Kunitz, in which the conditions for the enzyme activity determinations were not defined precisely enough.

The relationship of trypsin and chymotrypsin in Pancypsin was not determined before the experts' mission started.

The Quality Control Expert succeeded to introduce the new methods in cooperation with the specialists of ECAEM.

The short description of Armour method of the total proteolytic activity determination is annexed (5.27).

The Armour method was carried out using the haemoglobin reference standard and later on the haemoglobin working standard, produced in ECAEM. Its quality was not known and the content of nitrogen had to be determined before it was used for assay.

Practically all checked batches of Pancypsin have met the requirements of the world market. It was found that the total proteolytic activity was approximately 2 500 Armour units per mg of Pancypsin.

It was found that Pancypsin contained proper amounts of trypsin and chymotrypsin as required by the international market, too. Approximately 2 500 USP units of trypsin and approximately 500 USP units of chymotrypsin were obtained per one mg of Pancypsin. The requirement rate of trypsin : chymotrypsin is 5 : 1.

Annexed are (5.28) the photocopies of the relevant pages of the complete quality control procedures for chymotrypsin (pp. 216 and 217) and for trypsin (pp. 1104 and 1105), taken from USP XXI, 1985.

An additional problem was the lack of a s[illegible]ble spectrophotometer in ECAEM for the determination of trypsin and chymotrypsin activities. The quality control methods were modified and thus the difficulty overcome, but the purchase of a suitable spectrophotometer for future work in ECAEM was recommended (4.1.4.2.4 and Annex 5.30).

The Quality Control Expert provided the ECAEM specialists with the detailed papers on the above Armour and USP XXI methods.

The results obtained in ECAEM for the total proteolytic activity and specific activities of trypsin and chymotrypsin in Pancypsin, were approved by the Quality Control Department of "Galenika" Works in Belgrade, after the Quality Control Expert's return home (Annexes 5.31.1 and 5.31.2). The determination of the other characteristics of Pancypsin bulk purity, such as the contents of sulphate, ash and humidity, were carried out in ECAEM, except for the bulk density. That test was introduced by the Quality Control Expert, as well. The specification of Pancypsin on the basis of all parameters under the requirements for medical use was prepared and annexed (5.32).

3.6.2.2 Trypsin in Bulk

The USP XXI method for the determination of the specific proteolytic activity of trypsin (Annex 5.28) was introduced in ECAEM. The specific reagent, N-benzoyl-L- arginine ethyl ester hydrochloride (BAEE), was used as a substrate and USP Crystallized Trypsin Reference Standard as a reference standard. It was found that the trypsin of ECAEM has the activity that meets the requirement of the USP XXI, i.e. not less than 2 500 USP trypsin units in one mg of the product.

3.6.2.3 Chymotrypsin in Bulk

The USP XXI method for the determination of the specific chymotrypsin proteolytic activity (Annex 5. 28) was also introduced in ECAEM.

The specific reagent used as a substrate was N-acetyl-L-tyrosine ethyl ester (ATEE) and USP Chymotrypsin Reference Standard was the reference standard.

Five batches of chymotrypsin, produced in ECAEM, were checked and it was found that all of them met the requirement of the USP XXI, i.e. not less than 1000 USP chymotrypsin units per mg. The activity of ECAEM chymotrypsin was very high, approximately 1500 units per mg of the product.

The Quality Control Expert advised the ECAEM specialists that other testings of trypsin and chymotrypsin should be necessary under the requirement of the USP XXI. These included the solubility test, microbial limit, loss of drying, residue on ignition, content of chymotrypsin in trypsin and content of trypsin in chymotrypsin.

3.6.2.4 Pancreatin in Bulk

The Quality Control Expert introduced the FIP methods of activity determination of amylase, protease and lipase in pancreatin. During the introduction of these methods some difficulties arose, especially due to the lack of proper laboratory glassware and gear for lipase activity determination. In cooperation with the ECAEM specialists the method was modified and all other problems overcome.

The ECAEM pancreatin contained all three enzymes quoted. The activities were expressed in international FIP units. Annexed are (5.29) the photocopies of the relevant pages of the complete assay methods for pancreatin (pp. 72 to 84) , taken from the book, R. Ruyssen and A. Lauwers, Pharmaceutical Enzymes, Scientific Publishing Co., Gent, Belgium, 1978.

Introduced was determination of fat content in pancreatin, too. The method included the extraction by ether, and was carried out manually because of absence of the Soxhlet apparatus for extraction (4.1.4.2.4 and Annex 5.30). The fat content in ECAEM pancreatin was approximately 8%, which is slightly more than the FIP requirement of maximum 5% of fat.

3.6.2.5 Pepsin in Bulk

The FIP method of pepsin activity determination was introduced in ECAEM. Six batches of pepsin, prepared in the ECAEM Research Laboratory, were checked. High activity was found, approximately 4 FIP units per mg of the product. Annexed are (5.29) the photocopies of the relevant pages of the complete assay method for pepsin activity (pp. 87 to 94), taken from the book, R. Ruyssen and A. Lauwers, Pharmaceutical Enzymes, Scientific Publishing Co., Gent, Belgium, 1978.

The performing of the other tests of pepsin quality was advised to the ECAEM specialists, especially the pH checking. Pepsin is the most stable at pH 4 to 5.

3.6.2.6 Dry Bile in Bulk

According to the Quality Control Expert, the existing quality control method of dry bile in ECAEM should be changed and another, new, method introduced. It was not possible to do it during the Expert's stay in the field, because of the lack of needed reagents. The ECAEM specialists were provided with technical papers on the new quality control methods, for future use. The Quality Control Expert discussed the whole problem with the ECAEM specialists.

3.6.2.7 Cytochrome C

Cytochrome C is a completely new product and could not be found in any pharmacopoeia.

As cytochrome C contains and iron ion in the molecule, the assay of iron by spectrophotometric method, as used in the organic chemistry, was proposed in ECAEM. Such a method was not realized, because a reference standard and reagents needed were lacking.

3.6.3 Quality Control of the Raw Materials

3.6.3.1 Assay Methods of the Pancreatic Glands

Pancreas represents one of the main raw materials in ECAEM. A satisfactory output of enzyme preparations can be obtained only through proper treatment of pancreases, cleaning them immediately after slaughter and deep freezing them. Enzyme activity determination in the pancreatic glands, as the starting raw material, is of the same importance as is proper storage of glands in deep freeze. The FIP methods for enzyme activities determination of amylase and protease were introduced during the Quality Control Expert's mission in ECAEM. The lipase activity remained to be carried out in ECAEM in the future, after arrival of reagents and gear needed for the assay.

3.6.3.2 Checking of all Chemicals

The Quality Control Expert discussed and suggested to the ECAEM Director and specialists the performance of the routine checking of raw materials from batches of various products including all chemicals entering the store-house. This should assure good quality and increase the ECAEM productivity.

3.6.4 Microbiological Examinations in ECAEM

Microbiological examinations of final products in vials were carried out in ECAEM, and the results satisfied the requirements of the leading pharmacopoeias.
In order to ensure the quality of final enzyme products, the determination of bacterial purity of all bulk enzyme products was introduced by the Quality Control Expert. The batches of Pancypsin in bulk, checked on such a way, meet the world market requirements. The start of the new "Rota" line trial production was linked with a very strict system of microbiological control.

3.6.5 Quality Control Sample Technique

The sample technique was explained to the ECAEM specialists and they were provided with the suitable written instructions.
In the production of pharmaceuticals, every item in a batch should be checked. A sufficient number of samples must be treated to obtain a suitable degree of reliance on the result of the analysis.

3.6.6. Stability Checking

The quality of drugs should not change until the expiration date, if stored under proper conditions. Stability control tests are done by using an assay method specific enough to detect any active substance changes as well as some deterioration products. The Quality Control Expert discussed with the ECAEM Director the importance of introducing the stability control assays for enzyme products.

3.6.7 Instructions to ECAEM Specialists by the Quality Control Expert

In the course of the introduction of new quality control methods, the local personnel was trained simultaneously in the new methods, as well as in the preparation of reagents and in the maintenance of all parameters needed for the determination of the enzyme activities, such as temperature, pH, time, substrate concentracion, etc. Only under the mentioned conditions in the quality control procedure, the enzyme activity can be defined. Local personnel was trained also in the preparation of samples of frozen glands in order to determine the enzyme activities. A training programme for the personnel who are to work in the production of sterile enzyme in vials, was prepared as well. It included duties and responsibilities of all workers, as well as all sanitary-hygienic measures for securing the standard quality of drugs.

The ECAEM specialists were trained how to run the daily quality control protocols, too.

3.7 Project Second Phase Preparatory Work Done in ECAEM

The Project Second Phase draft preparatory work was done in ECAEM before the experts' stay in Ulan Bator. They based all the Project Second Phase products and capacities on the UNDP/UNIDO Project DP/MON/80/004 Feasibility Study "Establishment of the Pilot Plant for Processing of Biochemical Products", POLYTECHNA-SPOFA, Prague, 1981.
The National Counterpart agreed to discuss, and reviewed, all the matter with the CTA and other responsible persons later on, after completion of the Project First Phase (4.1.6).

4 RECOMMENDATIONS

4.1 Immediate Actions

4.1.1 Run and Completion of the "Rota" Line Trial Production

According to the Project Work Plan and the Mission Work Programme (5.2.1.), the trial production of enzyme products on the new "Rota" line were to be done in six weeks. As it sterted on 11 May 1987, it will be completed by 20 June 1987 (3.4.6 and 3.4.6.2). After the experts' departure from Ulan Bator, the Counterparts are taking care of the production (3.4.6), including the daily production capacity of Pancypsin, the weight of filled vials, the sterility control (3.4.6.1.1) according to the microbiologist Work Plan (5.15), and the toxicity and pyrogen control (3.4.6.1.3) according to the pharmacologist Work Plan (5.17).

4.1.2 Additional Equipment Needed for the "Rota" Line Reguler Run

The following equipment items are to be purchased immediately for the "Rota" line regular run:

4.1.2.1 Air-Conditioner

An air-conditioner has to be installed in the sterile room as soon as possible, as a necessity for the improvement of work conditions there (3.4.1 and 3.4.6.1.2).

4.1.2.2 Compressor

A new compressor, with a capacity big enough to supply with compressed air all the "Rota" line machines at once, is badly needed immediately. It has to be placed outside of the production rooms (3.4.1 and 3.4.4).

4.1.2.3 Ultraviolet Lamps

The room with freeze-drying machines has to be equipped with ultraviolet lamps, to improve the conditions of conveyance of the "Rota" line filled vials,from sterile room into the freeze--drying machine (3.4.1).

4.1.2.4 Autoclave

An additional autoclave is very important for regular sterilization of worker gowns, and gear for preparation of sterile Pancyosin solution. The existing autoclave in ECAEM is of a too small capacity. This is the reason why a purchase of an additional autoclave as soon as possible is strongly recommended (3.4.1 and 3.4.6.1.1).

4.1.3 Report on Findings upon Setting into Operation and on the Trial Production of the "Rota" Line

Such a report has to be prepared after the completion trial production.
The CTA will collect all the relevent data from the responsible ECAEM staff, and will thoroughly discuss the matter with the ECAEM Director and specialists. In agreement with the ECAEM Director, both of them together (the CTA and the ECAEM Director) will prepare their report on findings upon initiating the operation and on the trial production of the "Rota" line.

4.1.4.1 Carrying out of all the Quality Control Activities

Following the Quality Control Expert's advice, quality control of raw materials, including glands and chemicals, and of all final products are to be done constantly. The quality control methods introduced during the experts' mission are to be used continuously (3.6.2, 3.6.3, 3.6.4 and 3.6.5).

4.1.4.2 Improvements of the Existing Conditions in the ECAEM Quality Control Unit

It is recommended to provide as soon as possible all reagents and gear needed, and to take all other measures, to ensure the regular run of all quality control methods introduced in ECAEM during the experts' mission:

4.1.4.2.1 "Pro Analysi" Grade Reagents

The highest purity grade, "pro analysi", reagents in the quality control activities in ECAEM should be acquired. All needed reagents have to be purchased (3.6.1).

4.1.4.2.2 Highest Substrate Purity

The highest substrate purity for quantitative assay of enzyme activity should be required. It is advisable that all needed substrate be of such purity (3.6.1).

4.1.4.2.3 Reference Standards

The use of reference standards is vera important in the course of activity determination of enzyme preparations. It is therefore suggested to ECAEM to order all the standards needed, and also to prepare the national (or working) standards (3.6.1).

4.1.4.2.4 Apparatus and Glassware Needed for the ECAEM Quality Control Unit

The Quality Control Expert prepared together with his National Counterpart a list of selected apparatus and glassware needed for the quality control improvement in ECAEM (3.6.1, 3.6.2.1 and 3.6.2.4). The list is annexed (5.30). It is recommended to purchase immediately all that is possible from this list, and the rest to be procured by the Project Second Phase (4.2.3.3).

4.1.4.2.5 Manuals and Current Pharmacopoeias*

Manuals and some well known current pharmacopoeias, especially USP XXI, have to be purchased, and located in the Quality Control Unit (3.6.1). They are listed in the Annex 5.31.1.

4.1.4.2.6 More Space for the Quality Control Unit in ECAEM

It is strongly recommended that the Quality Control Unit be expanded as soon as possible, after the Quality Control Expert's suggestions (3.6.1).

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* The procurement of the International and European Pharmacopoeias as well as the Pharmaceutical Enzymes is also recommended.

4.1.4.2.7 Larger Staff in the Quality Control Unit in ECAEM

According to the Quality Control Expert's suggestions, larger staff is urgently needed in the Quality Control Unit in ECAEM, and it is advisable to employ new people as soon as possible (3.6.1).

4.1.5 CTA's Report on Completion of the Project

On the basis of the experts' joint report of the mission, the report on findings after starting operation and on the trial production of the "Rota" line (4.1.3), and on carrying out all of the quality control activities according to the Quality Control Expert's suggestions (4.1.4.1), the CTA will prepare the report on completion of the Project. In his report he will discuss as well the Project First Phase achievements.

4.1.6 Elaboration of Draft Project Document for the Project Second Phase, DP/MOR/86/C01

The CTA, the ECAEM Director, an UNIDO representative and maybe some other responsible persons, will take part in elaboration of the Project Second Phase Document, after the completion the Project First Phase terminal report (3.7).

4.2 Long Term Activities

4.2.1 Full Capacity Run of the "Rota" Line

After completion of the trial production, the "Rota" line has to run continuously with full capacity of the line. To ensure this, efforts are needed to sell Pancypsin in vials. The Ministry of Health control would facilitate that. Use the line for trypsin and chymotrypsin finalization in vials, too. Have it for serum gonadotropin in vials production for the veterinary use as well (4.2.2.1).
A new freeze-drying machine will be needed to balance the production capacity of the line (3.4.1 and 4.2.3.3).

4.2.1.1 "Rota" Specialist's Control of the Line Operation in ECAEM

It is strongly recommended to arrange with the "Rota" Company periodical checking of the sterile line operation by the Company specialist. This is the best way to ensure the long life of the line and the spare parts stock. Try to arrange the stay of the "Rota" specialist in ECAEM for a month once a year.

4.2.2 ECAEM New Products Implementation in the Larger Laboratory Scale Production Capacity

A continuous production of new products in ECAEM is recommended as soon as possible, such as pancreatin, pepsin from pig and pepsin from bovine stomach mucose, peptone, and some others (3.5.4). Start with the usual laboratory scale capacity and try to enlarge it to the larger laboratory scale soon. This is the way for the achievement of a bigger, pilot plant production capacity. Do not forget the principle of gradual enlargement of the production capacity, step by step. The same rule has to be observed concerning all the ECAEM products.

4.2.2.1 Serum Gonadotropin Raw Material Collection

It is strongly recommended that collecting of the pregnant mare blood be started as soon as possible. The serum gonadotropin production depends primarily on a successful arrangement of collection of a reasonable amount of such a blood. The procedure for the serum gonadotropin production is rather similar and not complicated (3.5.4.4). Needed is that ECAEM have this production running in a capacity big enough for the local needs and for export. The new "Rota" line production capacity will be fully employed in such a case, and this is very important for ECAEM (4.2.1).

4.2.3 Preparatory Work for the Project Second Phase

4.2.3.1 Choice of the Project Second Phase Products

Not too many products are recommended for the beginning of the Project Second Phase, maybe six to eight products, but no more. Choose the products with routine technology in ECAEM, with raw materials available in sufficient quantity and with real sales possibility both on the local and world market.
The well established ECAEM products, Pancypsin, dry bile, chymotrypsin, trypsin , are recommended in any case. After that, the products with the technology elaborated on the laboratory scale such as pancreatin, pepsin from pig stomach mucose, maybe pepto and pepsin from bovine stomach mucose. The reasons of the produ chosen for the Project Second Phase are explained briefly in Annex (5.33).
The next step will be new technology development such as for blood processing and bile acids.
In any case the new multipurpose pilot plant will have a possibility of easy changes of production technology, using a f standard equipment items for extraction and purification of animal sources.
All the products mentioned will be produced in the final form of substance, in bulk. Only Pancypsin, chymotrypsin and trypsir can be made as well in the form of final sterile drugs, freeze--dried in vials, using the existing "Rota" line.

4.2.3.2 Economic Survey of the Project Second Phase Products

The necessary economic survey of all products of the Project Seconf Phase is needed as soon as possible. This is important to ensure the sales on the world market, and also for a better evaluation of the Project Second Phase results.

4.2.3.3 Key Equipment for the Project Second Phase

Following the idea of easy changes of production technology in the new multipurpose pilot plant, well known standard equipment items can be suggested:

- Centrifuges and separators,
- Evaporator,
- Spray-dryer and
- Freeze-dryer.

A well known supplier of separators and centrifuges, and especially of evaporator Centri-Therm, Type CT-6, is the "Alfa Laval" Company, Sweden, and of spray-dryers is the "Niro Atomizer" Company, Denmark. The production of bioactive substances from animal sources in ECAEM is recommended primarily, and a choice of equipment has to be directed toward this end.

The final forms of drugs in ECAEM are limited by the existing "Rota" line for sterile drugs production in vials. The acquisition of a new freeze-drying unit has only to balance the production capacity of the line (4.2.1).

Use the existing huge capacity Tableting Unit of the Pharmaceutical Factory in Ulan Bator for all sorts of pills needed in ECAEM.

The quality control apperatus and glassware listed in Annex (5.30) are necessary for ECAEM and it is strongly recommended to purchase them as soon as possible (4.1.4.2.4), even using the Project Second Phase funds.

4.2.3.4 Training of ECAEM Specialists Abroad

4.2.3.4.1 Fellowship

A fellowship programme for training abroad of key personnel in production, quality control, maintenance and management is to be provided at the first stage of the Project Second Phase, and given the high priority. Try to choose a similar plant abroad for such training, and arrange the programme for each trainee of not less than three months abroad. According to the experience gathered from previous fellowship programmes, a shorter training will be less satisfactory.

4.2.3.4.2 Study Tours

In addition, study tours for local top executives of the Project to be provided at the beginning of the Project Second Phase implementation.
Try to choose some well known enterprises, for instance "Organon" in Holland.

4.2.3.5 New Building Construction for the Project Second Phase

A new building has to be constructed by the Mongolian side, before the start of the Project Second Phase.

4.2.3.5.1 Arrangement of Premises in the New Building

Recently ECAEM asked a special UNIDO mission, consisting of an engineer technologist and a mechanical engineer, to assist in preparing the detailed drawings of the building. How to arrange the premises, piping and the secondary activities, such as steam, water supply, electricity, sewerage system, etc.
These experts have to be careful, because of a multipurpose nature of the new pilot plant. They have to leave the possibility of choice and changes, and not too fix the equipment and the products in premises, because all this will depend on the Project Second Phase Document, which will be prepared later on, after completion of the Project First Phase terminal report (4.1.6).

4.2.3.5.2 Cooling and Freezing Chambers in the New Building

ECAEM badly needs cooling and freezing chambers with enough space. This may be very costly and not too easy to make it in the new building. Advice is, to try to use the Meat and Canned Meat Plant cooling facilities in any case, whether ECAEM remains a part of it, or is separated from it (4.2.4). Make efforts for an arrangement with the Meat and Canned Meat Plant, because they hav a huge cooling space, excellent cooling and freezing chambers, with reasonably low temperature.

4.2.4 Relation of ECAEM to the Meat and Canned Meat Plant in Ulan Bator

ECAEM belongs to the Meat and Canned Meat Plant in Ulan Bator, as part of this enterprise . Now, they are making efforts to become independent firm, especially because of the new pilot plant. The production of bioactive substances, mainly enzymes, differs substantially from a slaughterhouse production (what is more or less the Meat and Canned Meat Plant in Ulan Bator). As an independent firm, ECAEM will have more possibility of selling the products, and maybe to keep the rules and regulations of drug production, as arranged by the Ministry of Health (4.2.5).
On the other hand, the advantages of close relations with the slaughterhouse are the easy supply of the raw materials, what is one of the prime need in the enzyme an other bioactive substance production, and secondly sharing the cooling facilities, what is very important as well (4.2.3.5.2). The Meat and Canned Meat Pla in Ulan Bator possesses excellent cooling facilities of huge capacity.

4.2.5 Ministry of Health Control of Enzymes Produced in ECAEM and Used as Drugs

ECAEM belongs to the Ministry of the Light and Food Industries, as part of the Meat and Canned Meat Plant. In case of separation from the alsughterhouse (4.2.4), ECAEM will remain under the same Ministry.

The products of ECAEM are mainly drugs and as such they have to be controlled under the regulations of the Ministry of Health. Checking of ECAEM products by the State Drug Control Laboratory of the Ministry of Health is an advantage, because of easier sales of these products. The Ministry of Health is responsible for selling of drugs in Mongolia.

There are three possible levels of such control:

- Administrative control only,
- Control of sterility and safety and
- Full quality control.

This is usual in other drug producing enterprises in the world, especially the WHO control, which ensures easy disposal of the produced drugs on the whole world.

4.2.5.1 Pharmacological and Clinical Examinations of the Project Second Phase Products

The Ministry of Health can ensure the necessary pharmacological and clinical examinations of all products of the Project Second Phase, used as drugs. Such examination is needed and every new drug may be used and sold only with positive pharmacological and, later, clinical certificate. Pharmacological examinations can be costly, and clinical examinations costly and long-lasting. In spite of all, these examinations have to be done.

4.2.6 UNIDO Arrangement of the Project Second Phase

There are two possibilities of UNIDO arrangement, subcontract and individual experts engagements. Both possibilities have some advantages.

4.2.6.1 Subcontract

In case of subcontract, longer relations will be ensured with the subcontractor, advice and help to ECAEK will be rendered. The project will be fixed from the beginning and any change in the project later on will be hardly possible.

4.2.6.2 Individual Experts Engagements

If individual experts be engaged, they will be more interested in the project (because they will be better paid). Changes during the realization of the project will be easier, such as changes of products, capacities, etc.

5 ANNEXES

UNITED NATIONS

2.d

dr. Z. Caizer/rl

cl. Ms. A. Tcheknapotian-Asenbauer

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO

29 May 1986

JOB DESCRIPTION

DP/MON/82/002/11-01

Post title Industrial Biochemist (Chief Technical Adviser)

Duration 4 months

Date required 1 September 1986

Duty station Ulan Bator, Mongolia

Purpose of project To develop technologigal capability, research and development of suitable technology and utilization of locally available animal by-product for the preparation of sterile enzyme products (trypsin, chymotrypsin, pancreatin and gonadotropin).

Duties The Chief Technical Adviser in cooperation with the expert technologist and quality control expert will specifically be expected to carry out the following:

1. To supervise the production and quality control activities in the Experimental Centre of Applied Enzymology and Microbiology.

2. To prepare a detailed equipment specifications for updated improved technologies.

3. To assist in the organization of a study tour for selected supervisory personnel.

4. To assist the national counterpart in the selection of staff for individual fellowships.

5. To prepare a work plan with an implementation schedule of the project.

The Chief Technical Adviser will also be expected to prepare a final report setting out the findings of his mission and his recommendations to the Government on further action which might be taken.

..../..

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division
UNIDO, VIENNA INTERNATIONAL CENTRE, P.O. Box 300, Vienna, Austria

V.81-33106

Qualifications: Industrial Biochemist with extensive experience in production and quality control of sterile pharmaceutical preparations based on animal by-products.

Language: English/Russian

Background information: During the UNIDO Project Formulation Mission to the Mongolian People's Republic in April 1980, the problem of the development of a pharmaceutical industry and particularly the utilization of waste products from slaughterhouses was discussed with the Mongolian authorities concerned.

The pharmaceutical industry in Mongolia is still in the initial stage of development and is mainly confined to the production of dosage forms. The capacity of this production is insufficient to meet the country's requirements. At the same time a large range of indigenous raw materials is available in Mongolia for the production of pharmaceuticals for which there is a great demand in the country as well as in the international market. With this in mind the Government has given top priority to the project which has the aim to increase the production of drugs and some natural preparations by utilizing animal waste products from slaughterhouses. These wastes form one of the natural resources which Mongolia is abundant in. There are three slaughterhouses in Mongolia, all well equipped and possessing powerful regrigerators to keep waste products properly. About 2000 tons of waste products suitable for further processing are available yearly. The Government has already undertaken certain measures to establish a local industry for the utilization of these indigenous raw materials with the purpose of manufacturing pharmaceuticals and other biochemical produts. At the premises of the slaughterhouses in Ulan Bator, the Research Centre for Applied Enzymology and Microbiology has been set up. The Institute has already developed its own technology for the production of several sterile enzyme products such as Trypsin, Chymotrypsin and Pancypsin and others. It possesses a quality control laboratory and has some essential facilities for the production of sterile enzyme products. The next logical step is the establishment of a multipurpose pilot plant capable of manufacturing buochemical products according to the country's needs and export possibilities as well as to elaborate and develop technological processes with the aim of further scaling up and transferring them to industrial production level. The Government has requested UNDP/UNIDO for assistance in the establishment of the said plant and putting it into operation. At the first phase UNIDO has already provided preparatory assistance by sending a team of experts to carry out the techno-economic survey. The present project has been prepared based on the findings of that mission and reflects the results of discussions with the Government authorities concerned.

Due to the shortage of funds however that could be made available to the project from the on-going Country Programme as well as tremendous civil work which would be required to accomodate the proposed pilot plant, the above programme cannot be materialized in total for the time being. At the same time the Government has decided to start the project without further delay and to make available to the project, the premises of the Research Institute belonging to the Ministry of Light and Food Industry and premises of the plant in Ulan Bator which belongs to the same Ministry. The first phase of the project will cover the establishment of modern facilities for the production of sterile enzume products, strengthening of quality control and production of Gonadotropin in bulk.

The project is the first stage in the programme to enhance the development of a local pharmaceutical industry based on the utilization of locally available raw materials. The production programme which is being proposed for this stage is based on the experience and results already achieved at the Centre for Applied Enzymology and Microbiology and envisages the manufacture of pharmaceuticals and enzyme products both for local consumption as well as for import. The production programme will be as follows :

Product:	Quantity per Year:
Trypsin	6 kg
Chymotrypsin	10 kg
Pancreatin	80 kg

In view of the great demand for serum Gonadotropin, the production of this durg for veterinary use should balso be considered. The technology for the production of serum Gonadotropin is now at the stage of development in Mongolia. After installation of some additional equipment and solvent recovery unit, the Centre will be able to produce up to 5 kg Gonadotropin in bulk and in final dosage form.

UNITED NATIONS

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO

JOB DESCRIPTION

DP/MON/82/002//11-02

Post title Expert Technologist

ration 4 month

Date required 1 September 1986

Duty station Ulan Bator, Mongolia

Purpose of project To develop technological capability, research and development of suitable technology and utilization of locally available animal by-product for the preparation of sterile enzyme products (trypsin, chymotrypsin, pancreatin and gonadotropin).

Duties

The Expert Technologist, in cooperation with the Chief Technical Adviser and the Quality Control Expert, will be expected to carry out the following:

1. To supervise the production activities in the Experimental Centre of Applied Enzymology and Microbiology.
2. To evaluate availability of energy, water supply and waste disposal.
3. To select technologies and to prepare a list of equipment with specification for the production programme.
4. To prepare a report on his mission.
5. To cooperate with the Chief Technical Adviser and Quality Control Expert in preparing the final report.

..../..

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division

UNIDO VIENNA INTERNATIONAL CENTRE P.O. BOX 300

Qualifications Industrial Biochemist with a good experience in production of steri' bioactive substances based on animal by-products.

Language English/Russian

Background information During the UNIDO Project Formulation Mission to the Mongolian People': Republic in April 1980, the problem of the development of a pharmaceutical industry and particularly the utilization of waste products fro slaughterhouses was discussed with the Mongolian authorities concerne

The pharmaceutical industry in Mongolia is still in the initialstage of development and is mainly confined to the production of dosage for The capacity of this production is insufficient to meet the country's requirements. At the same time a large range of indigenous raw er is available in Mongolia for the production of pharmaceuticals in the international market. With this in mind the Government has given top priority to the project which has the aim to increase the production drugs and some natural preparations by utilizing animal waste product from slaughterhouses. These wastes form one of the natural resources which Mongolia is abundant in. There are three slaughterhouses in Mo golia, all well equipped and possessing powerful refrigerators to kee waste products properly. About 2000 tons of waste products suitable for further processing are available yearly. The Government has alre undertaken certain measures to establish a local industry for the uti zation of these indigenous raw materials with the purpose of maufacturing pharmaceuticals and other biochemical products. At the premises of the slaughterhouses in Ulan Bator, the Research Centre for Applied Enzymology and Microbiology has been set up. The Institute has alrea developed its own technology for the production of several sterile enzyme products such as Trypsin, Chymotrypsin and Pancypsin and other It possesses a quality control laboratory and has some essential facilities for the production of sterile enzyme products. The next l ic step is the establishment of a multipurpose pilot plant capable o manufacturing biochemical products according to the country's needs a export possibilities as well as to elaborate and develop technologica processes with the aim of further scaling up and transferring them to industrial production level. The Government has requested UNDP/UNIDO for assistance in the establishment of the said plant and putting it into operation. At the first phase UNIDO has already provided preparatory assistance by sending a team of experts to carry out the techno-economic survey. The present project has been prepared based the findings of that mission and reflects the results of discussions with the Government authorities concerned.

Due to the shortage of funds however that could be made available to the project from the on-going Country Programme as well as tremendous civil work which would be required to accomodate the proposed pilot plant, the above programme cannot be materialized in total for the time being. At the same time the Government has decided to start the project without further delay and to make available to the project, the premises of the Research Institute belonging to the Ministry of Light and Food Industry and premises of the plant in Ulan Bator which belongs to the same Ministry. The first phase of the project will cover the establishment of modern facilities for the production of sterile enzyme products, strengthening of quality control and production of Gonadotropin in bulk.

The project is the first stage in the programme to enhance the development of a local pharmaceutical industry based on the utilization of locally available raw materials. The production programme which is being proposed for this stage is based on the experience and results already achieved at the Centre for Applied Enzymology and Microbiology and envisages the manufacture of pharmaceuticals and enzyme products both for local consumption as well as for import.
The production programme will be as follows:

Product:	Quantity per Year:
Trypsin	6 kg
Chymotrypsin	10 kg
Pancreatin	80 kg

In view of the great demand for serum Gonadotropin, the production of this drug for veterinary use should also be considered. The technology for the production of serum Gonadotropin is now at the stage of development in Mongolia. After installation of some additional equipment and solvent recovery unit, the Centre will be able to produce up to 5 kg Gonadotropin in bulk and in final dosage form.

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

UNIDO

JOB DESCRIPTION

DP/MON/82/002//11-03

Post title Quality Control Expert

Duration 4 months

Date required 1 September 1986

Duty station Ulan Bator, Mongolia

Purpose of project To develop technological capability, research and development of suitable technology and utilization of locally available animal by-product for the preparation of sterile enzyme products (trypsin, chymotrypsin, pancreatin and gonadotropin).

Duties The Quality Control Expert, in cooperation with the Chief Technical Adviser and the Expert Technologist, will be expected to carry out the followings:

1. To supervise the Quality control activities in the Experimental Centre of Applied Enzymology and Microbiology.
2. To evaluate the existing facilities for Quality Control of pharmaceuticals.
3. To evaluate the Quality of raw material available; and suggest sampling and procedures for testing.
4. To prepare guidelines for the establishment and implementation of a systematic control procedure throughout the manufacturing lines and for the evaluation of standards of raw materials, semi-finished and end products.
5. To prepare a list of necessary analytical equipment.
6. To prepare a report of his mission.
7. To cooperate with the Chief Technical Adviser and the Expert Technologist in preparing the final report.

..../..

Qualifications A chemist or pharmacist with wide knowledge of modern analytical methods and international standards related to sterile pharmaceutical preparations including bioactive substances from animal sources.

Language English/Russian

Background information During the UNIDO Project Formulation Mission to the Mongolian People's Republic in April 1980, the problem of the development of a pharmaceutical industry and particularly the utilization of waste products from slaughterhouses was discussed with the Mongolian authorities concerned.

The pharmaceutical industry in Mongolia is still in the initial stage of development and is mainly confined to the production of dosage forms. The capacity of this production is insufficient to meet the country's requirements. At the same time a large range of indigenous raw materials is available in Mongolia for the production of pharmaceuticals in the international market. With this in mind the Government has given top priority to the project which has the aim to increase the production of drugs and some natural preparations by utilizing animal waste products from slaughterhouses. These wastes form one of the natural resources which Mongolia is abundant in. There are three slaughterhouses in Mongolia, all well equipped and possessing powerful refrigerators to keep waste products properly. About 2000 tons of waste products suitable for further processing are available yearly. The Government has already undertaken certain measures to establish a local industry for the utilization of these indigenous raw materials with the purpose of manufacturing pharmaceuticals and other biochemical products. At the premises of the slaughterhouses in Ulan Bator, the Research Centre for Applied Enzymology and Microbiology has been set up. The Institute has already developed its own technology for the production of several sterile enzyme products such as Trypsin, Chymotrypsin and Pancypsin and others. It possesses a quality control laboratory and has some essential facilities for the production of sterile enzyme products. The next logical step is the establishment of a multipurpose pilot plant capable of manufacturing biochemical products according to the country's needs and export possibilities as well as to elaborate and develop technological processes with the aim of further scaling up and transferring them to industrial production level. The Government has requested UNDP/UNIDO for assistance in the establishment of the said plant and putting it into operation. At the first phase UNIDO has already provided preparatory assistance by sending a team of experts to carry out the techno-economic survey. The present project has been prepared based on the findings of that mission and reflects the results of discussions with the Government authorities concerned.

Due to the shortage of funds however that could be made available to the project from the on-going Country Programme as well as tremendous civil work which would be required to accomodate the proposed pilot plant, the above programme cannot be materialized in total for the time being. At the same time the Government has decided to start the project without further delay and to make available to the project, the premises of the Research Institute belonging to the Ministry of Light and Food Industry and premises of the plant in Ulan Bator which belongs to the same Ministry. The first phase of the project will cover the establishment of modern facilities for the production of sterile enzyme products, strengthening of quality control and production of Gonadotropin in bulk.

The project is the first stage in the programme to enhance the development of a local pharmaceutical industry based on the utilization of locally available raw materials. The production programme which is being proposed for this stage is based on the experience and results already achieved at the Centre for Applied Enzymology and Microbiology and envisages the manufacture of pharmaceuticals and enzyme products both for local consumption as well as for import.
The production programme will be as follows:

Product:	Quantity per Year:
Trypsin	6 kg
Chymotrypsin	10 kg
Pancreatin	80 kg

In view of the great demand for serum Gonadotropin, the production of this drug for veterinary use should also be considered. The technology for the production of serum Gonadotropin is now at the stage of development in Mongolia. After installation of some additional equipment and solvent recovery unit, the Centre will be able to produce up to 5 kg Gonadotropin in bulk and in final dosage form.

Translation from Russian

Work Programme, Project No. DP/MON/82/002,
Assistance to the Experimental Centre of
Applied Enzymology and Microbiology
(Trypsin, Chymotrypsin, Pancypsin)

Approved 11 February 1987
L. Damdinsuren,
The Deputy Minister of the Light and Food
Industry of the Mongolian People's Republic.

prepared by: Professor Oleg Šćedrov, Chief Technical Adviser,
Vladimir Vavra, Consultant Technologist, and
Milka Cobanovic, Quality Control Consultant.

	Description of Activities	Done by	Location	Timing, from - to
	1	2	3	4
1	Second mission of the Chief Technical Adviser and the first mission of consultants: - a Technologist and - a Quality Control Consultant.		Ulan Bator	30 January 1987 arrival of the CTA, O.Šćedrov, the Consultant Technologist, V.Vavra, and the Quality Control Consultant M.Cobanović.
2	Elaboration of this Work Programme	CTA, Consultants, ECAEM leaders	ECAEM	6 February 1987
3	Familiarization of the CTA and two consultants with production, quality control and scientific research activities in the Experimental Centre of Applied Enzymology and Microbiology.	- " -	- " -	3 to 15 February 1987
4	Familiarization with the state of the preparatory work for installation of new line for the production of enzyme preparations, delivered for the project DP/MON/82/002.	- " -	- " -	3 to 15 February 1987

1		2	3	4
5	Installation and adjustment of the new equipment	Consultants, Counterparts, "Rota" engineer	Biochemical Dpt., ECAEM	15 to 28 February 1987
6	Training of the ECAEM workers and specialists on the operation of the new equipment.	- " -	- " -	23 February to 15 April 1987
7	Organization of operation of setting in motion and trial production of the enzyme products.	- " -	- " -	1 to 30 March 1987
8	Report on findings upon setting into operation.	CTA, ECAEM Director	- " -	15 April 1987
9	Rendering of advisory services and recommendations on introduction of the bioactive substances from animal sources and improvement of the quality, quality control and standardization of the final products.	CTA, Consultant Technologist and Quality Control Consultant	- " -	Up to the end of the mission.
10	Elaboration of draft plan for the project document of the Project Second Phase, MON/86/001, "Pilot Plant Establishment for Processing of Enzyme Preparations".	CTA, the two consultants, ECAEM leaders.	Ulan Bator	

1	2	3	4
11 Mission reports of two consultants.	Two consultants	Ulan Bator	25 April 1987
12 CTA's report on completion of the project.	CTA	Ulan Bator	8 May 1987

Chief Technical Adviser — Oleg Šcedrov

Director of the Experimental Centre of Applied Enzymology and Microbiology — J. Tserendendev

Translation from Russian

Annex 5.2.2

Work Programme of the
Consultant Technologist
Vladimir Vavra
Project DP/MON/82/002
Duration: 4 months

Approved 11 February 1987
L. Damdinsuren,
The Deputy Minister of the Light and Food Industry of the Mongolian People's Republic

	Description of Activities	Timing, from - to
1	Mission of the Consultant Technologist	30 January 1987
2	Preparation of the Work Programme	6 February 1987
3	Familiarization with the state of preparatory works on the installation of the new line for production of enzyme products.	3 to 15 February 1987
4	Familiarization with the state of work in the field of production and scientific research in ECAEM.	3 to 15 February 1987
5	Cooperation in installation and adjustment of the new equipment	15 to 28 February 1987
6	Training of ECAEM workers on the operation of the new line for the production of enzyme products.	23 February to 15 April 1987
7	Cooperation in setting the line into motion and trial production of sterile products.	1 to 30 March 1987
8	Cooperation with CTA in preparation of the report on findings upon setting the new line in motion.	15 April 1987
9	Rendering of advisory service and recommendations on introduction of the bioactive substances from animal sources.	Up to the end of the mission.
10	Elaboration of draft plan for the project document of the Project Second Phase, MON/86/001.	
11	Consultant's mission report.	25 April 1987

Prepared by: Vladimir Vavra

Translation from Russian

Annex 5.2.3

Work Programme of the
Quality Control Consultant
Milka Čobanović
Project DP/MON/82/002
Duration: 4 months

Approved 11 February 1987
L. Damdinsuren,
The Deputy Minister of the Light
and Food Industry of the
Mongolian People's Republic

	Description of Activities	Timing, from - to
1	Mission of the Quality Control Consultant	30 January 1987
2	Preparation of the Work Programme	6 February 1987
3	Familiarization with the state of production, quality control and scientific research activities in the Experimental Centre of Applied Enzymology and Microbiology.	3 to 15 February 1987
4	Familiarization with the state of the preparatory works on the installation of the new line for the production of enzyme products.	3 to 15 February 1987
5	Installation and adjustment of new equipment.	15 to 28 February 1987
6	Training of ECAEM workers and specialists on the operation of the new line for the production of enzyme products. Evaluation of the quality of the existing raw materials and proposition of the methods for testing of the raw materials being used for the enzyme production. Evaluation of the existing specifications of the raw materials, semi-products and final products on the basis of international standards. Proposition of methods for sampling of the raw materials, semi-products and final products. Proposition of methods for testing of the stability of the enzyme products with the aim to confirm the validity. Evaluation of the existing equipment in the Quality Control Department and proposition of the purchase of the new equipment.	23 February to 15 April 1987

	Description of Activities	Timing, from - to
7	Organization of operation of setting in motion and trial production of the enzyme products.	1 to 30 March 1987
8	Cooperation with CTA in preparation of the report on findings upon setting the new line in motion.	15 April 1987
9	Rendering of advisory services and recommendations on introduction of the bioactive substances from animal sources and improvement of quality control and standardization of final products.	Up to the end of the mission.
10	Elaboration of draft plan for the project document of the Project Second Phase, MON/86/001.	
11	Consultant's mission report.	25 April 1987

Prepared by: Milka Čobanović

Annex 5.3

Various Persons Met

In addition to the persons met during the CTA's first mission in Ulan Bator in 1985, and listed in the Mission Report of 19 June 1985, the following persons were met during the CTA's and the two experts' stay in the field from 30 January to 19 May 1987.

5.3.1 Mr. Javier Péres De Cuéllar, Secretary-General of the United Nations, Ulan Bator, Mongolia, 16 May 1987.

5.3.2 Mr. J. I. Litoukhin, Resident Representative, UNDP, Ulan Bator, Mongolia, 2 February to 18 May 1987.

5.3.3 Mr. M. K. Polievktov, Chief Technical Adviser of the UNIDO Project DP/MON/84/001, Ulan Bator, Mongolia, 25 February, 7 March and 10 April 1987.

5.3.4 Mr. M. Gunsendorj, Head of the Department of the Ministry of the Light and Food Industry of the Mongolian People's Republic, Ulan Bator, Mongolia, 23 April and 11 May 1987.

5.3.5 Mr. P. Luvsan-Namjil, Manager General of the Pharmaceutical Factory, Ulan Bator, Mongolia, 25 February 1987.

5.3.6 Dr. Dandii, Director of "Biomed", the Institute for Production of Human Blood Derivatives, Ulan Bator, Mongolia, 26 February 1987.

5.3.7 Dr. C. Janchiv, Director of the Institute of Experimental Biology of the Mongolian Academy of Science, Ulan Bator, Mongolia, 5 March 1987.

5.3.8 Dr. B. Dashnyam, Deputy Director of the Institute of Experimental Biology of the Mongolian Academy of Science, Ulan Bator, Mongolia, 10 April and 15 May 1987.

5.3.9 Dr. G. Gombo, Director of the Scientific-Experimental Centre of the Foodstuff Industry, Ulan Bator, Mongolia, 28 April and 6 May 1987.

5.3.10 Mr. K. Kabden, Pharmacologist of the Experimental Centre of Applied Enzymology and Microbiology, Ulan Bator, Mongolia, 4 February to 15 May 1987.

5.3.11 Mr. Oldoh, Mechanical Engineer of the Experimental Centre of Applied Enzymology and Microbiology, Ulan Bator, Mongolia, 4 February to 19 May 1987.

5.3.12 Mr. N. Khodjnoi, Electronical Engineer of the Experimental Centre of Applied Enzymology and Microbiology, Ulan Bator, Mongolia, 4 February to 15 May 1987.

5.3.13 Mr. K. Randjelovic, "Rota" Firm Engineer, Ulan Bator, Mongolia, 1 to 12 May 1987.

Annex 5.4

Perticipation of the CTA in the Formuletion of the new Project Document DP/MON/86/C08, "Development of Biotechnology and Genetic Engineering".

As requested by Mr. J. I. Litoukhin, Resident Representative UNDP in Ulan Bator (5.3.2), the CTA checked on 16 February 1987 draft Project Document DP/MON/86/C08, of the Institute of Experimental Biology of the Mongolian Academy of Science. A visit was paid to the Institute on 5 March 1987.

The CTA studied a new improved version of the Project Document on 9 April 1987, upon a request by the Project National Counterpart dr. B. Dashnyam, Deputy Director of the Institute (5.3.8). On 10 April 1987 a meeting was held in the UNDP Office with Miss D. Elez, Assistant Resident Representative UNDP, Mr. M.-K. Polievktov (5.3.3) and dr. Dashnyam (5.3.8). The Project Document was discussed and the final form of the Document was arranged. A separate discussion was held with dr. Dashnyam, whom the CTA gave additional suggestions, so that some parts of the Documet could be improved.

The biotechnological production of L-lysine, using microorganisms, is possible and needed in Mongolia. The hepatitis B vaccine made by the recombinant gene techniques is interesting, but it is not so easy to make it. In any case, it is useful to deal with this problem, so that more experience in this field be acquired and the solution of this problem of genetic engineering may be tried.

The Institute has good specialists, biologists, with scientific degrees and experienced to a high degree, but for solving the Project biochemists will be needed as well. This subject was discussed with dr. Dashnyam (5.3.8), too.

The planned Project, with UNDP contribution of $ 600 000.-, ought to be funds well invested and should considerably help the Institute in further progress.

Annex 5.5

Professor Oleg Ščedrov Ulan Bator, 15 May 1987

What is Biochemistry

The biochemistry is a science which investigates the phenomena of life by chemical methods. We can consider it as a part of life sciences. It is relatively young science.

Modern biochemistry was established primarily upon organic chemistry and biology. On the other hand we can say that it represents an extension or continuation of organic chemistry and biology. Without solid knowledge and understanding of organic chemistry it will be impossible to understand biochemistry. It is not enough to learn, to memorize. Biochemistry must be comprehended, understood chemically. To observe the phenomena of life through the eyes of chemists. That means to be good in biochemistry.

The substances obtained from the living creatures were systematized first time by Berzelius in the beginning of the nineteenth century. He gave them the name of organic substances. So it was the origination of the organic chemistry. Already then it was considered that organic substances consisted of carbon, hydrogen, oxygen and nitrogen and that is what makes the essential difference from the inorganic world. Today we extend this list in some degree, primarily adding phosphorus and sulphur.

There existed a great problem at the time of the origination of organic chemistry. The vitalists, followers of the theory of the life force, vis vitalis, stated that the compounds in the plants and animals originated in another way than the inorganic substances. They said that rough and simple chemical forces cannot act in a living organism. Accordingly, the organic substances differ from the inorganic ones because for their existance a special life force is necessary. It looked that it was impossible to get an organic substance in an artificial way, by the chemical processes. The ideas about the nature of this hypothetic force were vague and foggy. This was a theory which impeded further development of science.

But, Wöhler succeeded in geting urea from ammonium cyanate in the thirties of the last century:

$$NH_4CNO \longrightarrow H_2NCONH_2$$

and so obtained an organic substance from an inorganic one. This experiment spoke without doubt against the vitalists. Nevertheless, there had to pass next twenty years to bring along new proofs, and finally to pull down the bad theory. The vitalistic theory disappeared slowly under the evidence of the gathered facts, and not as a consequence of any single brilliant experiment. Similarly, slow changes were characteristic with other scientific theories, and also with those which are considered good today.

Let's go back once more into the last century, to see another conflict of standpoints. Pasteur and Liebig! Both of them were great, we admire both of them and appreciate them very much. In the fifties Liebig held the point of view that the alcoholic fermentation was a chemical process and that living microorganisms were not necessary. Pasteur asserted that alcoholic fermentation was connected with the living cells of Saccharomyces species. According to Pasteur those were organized ferments and differed from pepsin and amylase, which he called soluble ferments. At the first glance Pasteur was wrong, he impeded the development of science. But we must be reminded that in his time, more than a hundred years ago, it was not known that the microbes cause infectious diseases. It was directly to the merit of Pasteur that the correct attitude of mind about the infectious diseases and their causers, the microorganisms, predominated at that time. Pasteur just took a unified standpoint. We cannot criticize him too much, and to the contrary we shall highly appraise him, after we summed up all what he had done. To mention only rabies and anthrax, cholera of poultry, and his immunization vaccines. Let us finish this small trip into the past. After next fifty years, at the end of the last century, the dilemma disappeared when Buchner proved that dead and crushed cells of yeast fermented the sugars, too.

The rapid development of synthetic organic chemistry repressed for a long time any interest in natural compounds.

The organic chemistry is defined today as the chemistry of the carbon compounds.

Let us see what is the scope of biochemistry. It introduces us to the chemical ingredients of cells. According to the old division this is the descriptive biochemistry. In the last decades there existed a great interest for highmolecular natural compounds. The determination of the composition and structure of proteins and nucleic acids is in the focus of attention. But life is a dynamic phenomenon. Any description of chemical substances gives us only a static picture of the momentary status in a living cell. The study of chemical processes which take place continuously in a cell, make the field of dynamic biochemistry. Almost all substances from which the living cells are built up are in a continuous change. The characteristic of life is motion and all what were previously seen as the static forms, are in fact included in the chemical changes and movements. Thus, the biochemistry of today is dynamic before all. It is the metabolism, or exchange of substances, with various alterations and degradations of natural compounds in order to obtain chemical energy and to building up its own cellular substances. These chemical reactions are catalyzed by the enzymes and their study covers a large part of the biochemistry. The chemical regulation belongs to the dynamic biochemistry, too. Usually this function is performed by special substances, the hormones, which are made in glands with internal secretions.

It would be necessary to mention that the biochemistry includes the processes which occur in the so-called structures, too. As the cell structures (cell walls and coats) and also membranes (liquid bilayer, plasma membrane), are the chemical products, so the changes in them represent the biochemical reactions. The biochemistry is able to explain the physiological processes and penetrate more deeply into the essence, than could be done by the physiological approach only. From recent time yet there exist a saying that every a physiological problem can be ultimately turned into a biochemical problem.

The fundamental manifestations of life are reduced today to the biochemical processes, too. Let us consider for instance the molecular biology, then the chemical nature and methods of action of the hereditary agents. As a matter of fact this is only a new name for the field of biochemistry which tries to explain the fundamental phenomena of life on the molecular, biochemical, level. In recent years we came to understand much, but yet in this field there exist considerable unsolved questions. Recombinant DNA technology has revolutionized the study of the cell. Any region of the cell's DNA can now be excised with restriction nucleases and produced in virtually unlimited quantities by DNA cloning and then sequenced at rates of hundreds of nucleotides a day. As a result, many genes and noncoding regions of the eucaryotic genome have already been sequenced. The practical consequences of recombinant DNA (gene) technology are farreaching. Bacteria or yeast can be engineered to make a mammalian protein in virtually unlimited quantities, making it possible to analyze the structure and function of the protein or to use the protein as a vaccine or drug for medical purposes. Insulin and growth hormone as well as interferon production on such a way is well known. Now, you are trying to make the hepatitis B vaccine using recombinant gene technology. Chemical methods cannot explain some life phenomena. The bioelectric phenomena require other methods of investigation. Human consciousness for instance, cannot be handed by the natural scientific analysis.

And something more for our consideration. Every living creature, and you and me, constitute a metastable state which is in contradiction with the Second Law of Thermodynamics[x]. Entropy is the measure of the size of the disorder. A living organism represents a settled organization and the size of disorder is not increased in him. Obviously, until it is alive. After death the disorder rapidly start to increase, the entropy grows, and the thermodynamic principles will be satisfied.

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[x] The Second Law of Thermodynamics: The energy always goes in only one direction, from the point of higher state to the point of a lower state, or in simpler words, from a warmer body to a colder one.

The results of the biochemistry are applied to many related sciences.
All drugs as chemical substances influence the biochemical processes in the organism.

Biochemistry and biology consider the same subject, the living cell. So, they are sister sciences, but they have different approaches to the cell, biology from the living point of view, and biochemistry from the chemical standpoint. It seems that in the future, I hope in the near future, they both (cell biology and bicchemistry) will combine to make one single science. Untill then a close cooperation is needed between us (cell biologists and biochemists) in solving all the problems of the cell.

Annex 5.6

The experts' comments to the preparatory work for sterile enzymes production

After being acknowledged with the existing drawings for arrangement of sterile room for sterile enzymes production on 13 February, we have the following comments and recommendations:

1) To install the necessary sterilizing UV-lamps on the ceilings. These lamps should work overnight and over any break of work and must be switchable to normal light during working hours.
2) To paint all the plaster-walls inside sterile rooms with lacquer to avoid dusting and to enable washing of the walls. The same should be done with ceilings.
3) The walls should be free of any pipings or tubings to enable washing and sterilization of the whole space.
4) To install some sterile-air conditioner (ventilator with filter) to get some overpressure in the sterile room, avoiding so entry of any germs from outside; also to bring some fresh air for workers.
5) All necessary furniture in sterile rooms must be easily washable with smooth, lacquer-painted surface.
6) No openable or leaking windows, doors from sterile room only to the sterile corridor.
7) Our recommendations for the future: To turn the whole system so, that sterile room for filling is beside sterile washing loop for employees so, that they are coming straight into the sterile room. In this case no sterile corridor is necessary, system enables achieving of more precise working conditions and brings more free place for another activities. Needs new sewerage and steam and water supply. In connection with this to build the Leybold freeze-drying machines into thin wall so, that they are approachable either from sterile room for drying of sterile products or from behind for necessary maintenance.

Ulan Bator, 17 February 1987

Prof. Čedrov Oleg
Jovanović Milka
Vavra Vladimir

Annex 5.7

The comments on Rota documentation - Operating Instructions

The documentation for Rota line was sent from Rota firm on 17.3.87 and came to Ulan Bator on 25.3.1987. Some of the copies of the doucmentation are dated with rather late dates; the main drawing for the whole connected line carries date of 20.2.1987 (drawing K50 Z-6), which means, that it was not prepared at the time when Rota engineer should have been already in Mongolia.

The most serious thing is, that there are rather large differences in sizes of the individual apparatuses when compared with previously obtained documentation, the prospectusses, which were used when the line was being ordered and according to which the necessary preparatory work in the premises of ECAEM has been done. The greatest difference is in the width of the sterile tunnel unit type TU/11-300, now with additional specification.ST-1, which instead of previously declared 800 mm is now 1030 mm. This means that the already prepared opening in the wall between sterile room and preparatory room must be once again enlarged and adapted to the new size.

The sizes of washing machine Jiffy I differ as well, which should not matter so much if there had been beforehand some information about a connected device, which according to the drawing K50 Z-6, enlarges the width of the machine for another 900 mm on one side from the central axis. This device is for the first time specified in the drawing and it may be "additional Pump-over Agregate" which has not been ever specified before.

In the documentation for washing machine Jiffy I the page 6.9.5., Lay out of the Electrical Plant, is missing and there are mistakes in text and corresponding drawings. For example on page 9.2 the exact codes for parts to be operated with in points 2 and 6 are missing, on page 7.1 excentric axis LA34B mentioned there cannot be found in corresponding drawing 3.

In the documentation for sterile tunnel ST-1 is missing on page 5 and 6 the quantity of expelled air and air intake. First is there remarked, that the temperature of intake air must be approximately 20°C; being not informed beforehand it is not sure now how to arrange in premises of ECAEM the constant temperature of air. First is mentioned there the necessity of compressed air supply system with min. pressure of 3 atm. According to the page 15 there should be the handbook for operation and maintenance for computerised recorder. Not there.

The last part of the documentation is for fully automatic machine for filling and closing of vials, type FLR-50/GB. According to the prospectus for filling machines, this GB type serves for filling, stoppering and capping, therefore it does not correspond exactly to the two different machines FLR-50/G for filling and stoppering and FLR-50/B for feeding and grimping of aluminium caps, which were ordered and purchased. In this documentation is missing the complete drawing of the machine, to which there are made references on page 3 of the documentation.

This means that there are missing documentations for Vial filling and closing machine FLR-50/G (item C1 of the invoice) and Vial closing machine (itemD1) type FLR-50/B. Besides this, there is no documentation for Labelling machine type RE-50 (item E1) and Laminar-flow cleanroom unit (item C 17.1).

According to the findings (especially to the changes of sizes of equipment) we must ask once again, why the documentation had not been sent before any preparatory works at the ECAEM premises began.

Ulan Bator 3 April 1987

Vavra Vladimir
Expert Technologist

Short information on the function of Rota line equipment

The Rota line is working as a fully automatized equipment for washing, sterilizing and filling of the vials; completed with the crimping machine and labelling machine it represents a complete auto-automatized system for production of sterile products in vials.

The line consist of 3 main machines coupled together to form the line and 2 machines serving for finishing the work after necessary freeze-drying of the sterile product solution in vials.

The first machine standing in the clean room - <u>washing machine Jiffy I</u>, serves for washing of the vials after their transport to the workshop. The worker is only unpacking the vials and putting them on the filling chute of the machine. From there the machine is taking the vials automatically, turning them mouth-down and taking them to the washing unit. There the vials are washed with sprinkling tap water, destilled water, the remaining drops of water are then blowed out of the vials with compressed air, vials are turned mouth-upwards and are going automatically out fo the machine through transporter to sterilizing tunnel. The working speed of the machine is adjustable up to the capacity of 3600 vials per hour, and the speed is controlled electronically by special electronic card device. The machine has automatic control against possible overloading and safety device against possible opening of the machine during its running. It is equipped with pannel signalizing the good running of the machine as well as any possible disorder in running. The consumption of water represents amount of about 150 lt/hour, consumption of compressed air about 500 lt/min.

<u>Sterilizing tunnel TU/11-300 ST-1</u> This device is also automatically running, being controlled by the computerized recorder, which is recording all the datas about correct running of the device through the whole shift, enabling so the necessary control in any case of problems with sterility of the final products. The vials are going into the sterilizing tunnel after leaving the washing machine and are going through the tunnel, where they are sterilized with high temperature of about 350°C for 20 minutes, during their transport through tunnel. At the end of the tunnel the vials are cooled with fresh, sterile, cooling air to the temperature of about 25°C and are transported on transporter into the sterile room, where they are coming automatically to the filling machine. The sterilizing

tunnel is connected hermetically to the wall of sterile room. The machine is equipped with pannel signalising the correct function of all parts of the equipment, or any possible disturbence or disorder. Before installation it is necessary to prepare connecting tubing for intake of cooling air (which should be taken in some distance from the machine and should have constant temperature of about 20°C both in winter or summer period) and connecting tubing for exhaust of not air from the machine. Both tubings have diameter of 360 mm; the exhaust of hot air should be led out of the room, in order not to increase temperature inside the working area. This machine should be as well connected to the compressed air supply for stetching of the transport-belt.

Filling machine FLR-50/G is placed inside the sterile room and is moreover covered by the Laminar-flow unit, which is blowing sterile air over the whole machine, protecting so the filling process against any possible contamination. The vials are coming to the machine on on transporter from sterilizing tunnel and are collected on a feeding chute of the machine. From there they are taken by continuously rotating feeding screw to the star-wheel, where each vial is first filled with prepared sterile solution of product via dosing pump with filling needle. After filling, a sterile resin stopper is placed automatically on each vial from feeder and inserted partially into the vial opening. After this half-stoppering the filled vials are going out of the machine onto the collecting chute. From there they are taken by the worker and transported on appropriate tray through connecting window to the freeze-drying Leybold machine.

After freeze-drying the vials are automatically fully closed, being still in the Leybold machine and then they can be transpotred to the clean room, to the crimping and labelling machines. There is no documentation for the filling machine FLR-50/G.

Crimping machine FLR-50/B There is no documentation for this special machine, which is automatically placing aluminium caps onto each vial and fixing them on the vials by crimping of the edges. The vials from the Leybold are transported by worker onto feeding chute of the machine from where they are taken for further processing automatically by the transport-screw of the machine. The Alu caps are filled into bowl-feeder, where they are automatically sorted. By feeding rail are then the Alu caps transported upon the vials and crimpped on each vial by means of 2 pressure-rollers. After crimping the vials are transported from machine onto collecting chute, from where they are taken for further

processing.There may be done some possible connection to the last machine, the labelling machine, but having no technical documentation it cannot be said surely.

Labelling machine for auto-adhesive labels RE-50. There is as well no technical documentation for this machine. On this machine is done the labelling of filled and closed vials and the machine is as well working automatically. The viels are transported from feeding chute by means of feeding worm gear to the star-wheel. The machine is equipped with changeable printing head, where the correct datas concerning the name of product, number of batch and other informations are prepared before starting of the work. The selfadhesive labels are first printed by the printing head and then transported (still sticking to the transporting release paper) to the star-wheel with vials.The label is automatically transferred onto the vial, pressed on by means of pressing roller and the labelled vial is leaving the machine onto the collecting chute. From there they are collected by worker for final packing. The height between vial bottom and label can be adjusted by a fixing screw.

This information have been prepared on request from Mongolian side.

Ulan Bator 22 April 1987

Vavra Vladimir
Expert Technologist

9

Quality Control Expert's Preliminary Report

The production of the sterile products, according to the WHO accepted standards and the pharmaceutical ethics, can be done only if the premises for production of the sterile products are provided with the following:

- filtered and sterile air,
- higher pressure than in the surrounding areas,
- clean passing room and other rooms such as:
  a - wash and decontamination room,
  b - cloak-room for putting sterile clothes, masks, glo- and shoes on.
- To reach the highest level of aseptic conditions by putting the relevant equipment into specially isolated areas with stream of filtered and steriled air Laminar Flow.
- To equip the production areas with suitable light sources and equipment for maintaing temperature and humidity conditions.
- To avoid condensation of water-steam in the sterile areas, therefore these areas must have air-exchanging facilities which are to be fitted and maintained in such a manner that the microbiological, chemical or mechanical contaminations are avoided.
- Containers, equipment and other furniture should be made of such a material which enables easy desinfection.
- The floors, walls and ceillings should be made of impervious materials with a smooth surface. They should be free from cracks and open joints, and constructed in such a way that easy and quick maintenance is possible in order to have clean conditions continuously.
- The equipment should be installed in such a way that its repair or replacement could be done outside the sterile area. During repair, products, equipment and premises should be protected and if there are no aseptic conditions, the area should be cleaned after the repair.

Ulan Bator, 16 February 1987

Milka Cobanovic

Annex 5.10

Recomendations on correct preparation of sterile room for production of sterile ampoules

The part of area,designed for production of sterile ampoules must be sterile. Therefore all the raceways for pipes and cables must be eliminated. Windows should stay shut or even be sealed. All edges should be avoided. Walls, ceiling, windows and doors will have to be painted with washable paint, the joints of floors,walls and ceilings should be rounded.The floor should be covered with linoleum with the least possible junctions.

The whole production area should have a filtered air ventilation system with at least 10 changes per hour.

The utmost precaution should be taken to avoid bacterial contamination both of the product and fo the equipment.

The personnel should be in good health, well aware of the rules, regulating the handling of sterile products, and must wear clean dresses, which will have to be changed daily.There should be two dressing rooms, one to take off the clothes worn outside and to put on overalls, the second room to take the latter off and put on those, worn within the sterile room only.

These clothes, which will have to be changed at least once every day, must be carefully washed, kept apart from other clothing and steam sterilized at 121°C for 30 minutes.

The rooms will have to be supplied with germicidal lamps which are lighted upon the workers leaving the place at the end of their shift,and remain in operation the whole night.

Production of water suitable for injectable use.
This water must be practically sterile (contamination not higher than 0,1 colonies/ml at the delivery point) and apyrogenous. To obtain this type of water a ion-exchange demineralizer and a continuos distiller is needed. Distilled apyrogenous water should be preserved and distributed so as not to allow microbial growth. Such aim can be attained when water is kept at a temperature of 80°C and not longer than 24 hours.

The containers within which the water is transported should be apyrogenous and sterile too.

UlanBator 10 March 1987

Production of sterile products and its obligatory control

In the production of sterile drugs basic conditions of correct work should be assured. They include suitable equipment, personnel and premises, as well as raw materials.

Requirements for good drug protection should be very strict in production of sterile products because that is the only way how to prevent eventual microbial contamination of drugs. It is therefore important to carry out all tasks concerning assurance of good quality from the very beginning of such production. The final quality control or quality control during the production process can not already satisfactory assure good quality of the final products.

Production of sterile drugs depends on good knowledge, experience and technological discipline of all personnel especially on those, who directly run the work on sterile machines. Duties and responsibilities of all workers should be clearly explained to them and should be recorded in written prescribtion or in other suitable way. The responsible personnel should take care of the quality of their work and of the right usage, maintaining and correct usage of the equipment.

The production personnel should be well acquainted with the organizing of work in the production cycle they are concerned with and they must do their tasks according to the production documentation, which must be written on each phase of the production.

The sterile production personnel should be avoided of direct contact with materials, or intermediate products during the whole period of their work.

The maintenance personnel, doing some necessary repairs in the sterile area is subjected to the same dissipline as the personnel from production. Tools or spare parts should be in case of their necessity sterilized. During repair, products, machines and rooms should be protected against possible contamination and in case of possible contamination the area should be cleaned after the repair.

The movement of personnel in sterile and clean areas should be minimized.

The premises should be free from all meterials and sterile rooms should not contain during technological procedures any unnecessary things like rejected packing material, cleaning agents, clothings, food,

Eating, drinking, smoking or nonhygienic acts are forbidden.

Protective clothes should be purposefull comfortable and made of material which could be sterilized.

The clothes should be changed from time to time; the clothes, shoes and head covering means should be changed whenever the person goes out and returns to the sterile area. Gloves should be changed when needed, but at least once in a shift.

For securing of declared quality of sterile products the producer must control the conditions of sterile rooms regularly in prescribed periods of time. In any case of changing the equipment or raw materials or even in change of some employees in the sterile production it is necessary to carry out the microbiological control more often, untill standardization of sterile conditions is reached.

Before and during the production of sterile drugs, routine microbiological control of air, equipment and clothing should be done.

Production documentation

The production documentation represents the basis for reliable work and limits the danger of errors. Documents should contain the single tasks and manners in which they should be carried out, following all phases of the production process. Documentation should prevent omitting of any phase of the whole process and can prevent any possible misunderstanding which is possible in oral communication only.

Each phase of production must be clearly formulated, supervised and directed according to the regulations.

The technological procedure must be dated by the competent manufacturing unit and contain:

The name, form, dose or strength (activity) of product

List of used raw materials and the list of packing materials.

Detailed description of all packing phases including printing of control number and expiration date.

Ulan Bator 27 April 1987

Sobanović Milka

Quality Control Expert

Annex 5.1.2

Recommendations and rules for correct production of sterile products.

To ensure sterility and good quality of the final sterile products it is necessary to keep all rules for correct sterile work.

1) All material, all surfaces which are coming into any contact with sterile solutions must be as well sterile. This means that it must be well packed and sterilized and remain packed untill it is used. When it is taken out of the packing there must be done no touch with hands on the surface which is coming into contact with sterile solution. All sterilized material must be protected from any contact with contaminated nonsterile air. Material for taking samples, or u used for filling on filling machine (the tubes,pump,needles of the machine) must be as well always sterilized before work.

2) Solution of enzyme must be prepared only the same day when sterile filtration is done, because of danger of a) pyrogenity,b) loss of activity. It is always better to work quickly to ensure good quality of product.

3) The water used for solving of product must be fresh distilled water; the same should be in case of water used for washing of vials. In no case can be used old water because of danger of pyrogen.

4) The sterile filtration is done on sterilized and covered equipment into sterilized flasks or other sterile equipment. After filtration, the flask with sterile solution is covered immediately with sterile piece of paper or other material, and must remain covered. In case of filtration with vacuum, the tube connecting the sterile flask with vacuum must be filled with some protecting material (usually cotton-wool).

5) During any transportation, even in sterile room, the flask with sterile solution must be covered. Taking of any samples is possible only with sterilized pipette, which is uncovered only just before usage and is not used once again in cases it is not sure they remained sterile.

6) Before filling of the sterile solution the filling device of the machine must be always sterilized, being packed before sterilization; during mounting of the machine care must be taken not to touch surface coming into contact with sterile solution.

7) During filling, in case that any stopper falls from the filled vial both, stopper and vial must be removed. The same in any case when it is not sure the vials are sterile - for example in case of some necessary arrangement of machine, remove all the vials which might have come into contact even with your breath.
8) Because of necessary transportation of filled, sterile, half-shut vials over nonsterile room to Leybold machines, it is better to remove all, which are badly covered with stopper.

The room with Leybold is now nonsterile room. It is necessary to solve this problem; now with installation of stronger UV lamp on ceiling, in future better to install Leybold into some thin wall, dividing sterile room from nonsterile room. (This was already recommended in first report).
9) The vials after freeze-drying are not yet compactly closed; therefore it is not possible to handle with them like with final product. They should remain covered and placed in some sterile or at least clean room, not lying and not being shaked with. Because of vaccum inside the vials some contaminated air could get inside.
10) The workers must know, that even after a very careful washing their hands are not sterile; therefore they must not get into touch with any sterile material. As well keep in mind that no organic surface can be 100% sterile.

<u>It is obligatory, that every new, unexperienced worker is well acknowledged with the correct work rules</u>.

Ulan Bator 14 May 1987

Annex 5.13

Ulan Bator, 11 May 1987

P R O T O C O L

on Delivery of Rota Filling Line Equipment

The consignment of Rota equipment for installation of filling line consisting of 9 wooden cases, purchase order No. 15-5-A1362 dt. 07/11/85, was sent by railway from West Germany on 28 Nov. 1986. It was delivered to Ulan Bator on 6 Jan. 1987. None of the cases had been opened before Rota specialist arrived.

The cases were opened on 3 May 1987 in presence of representatives of Rota firm (Mr.K.Randjelovic), Experimental Centre of Applied Enzymology and Microbiology (Mr.J.Tserendendev and Dr.J.Alimaa) and of UNIDO experts (Prof.O.Scedrov and Mr.V.Vavra). It was found that:

1) Case No.1 containing washing machine Jiffy I, Pump-over agregate, electrical heating and one set of spare parts, must have fallen hard during transportation. The legs of the machine went out of the fastening and went through the bottom-boards of the case. One leg became broken and the connecting-plug was damaged.

2) Case No.2 containing Sterilising tunnel ST-1 with spare parts, was as well damaged during transportation. The handles of the machine broke through the side-boards of the wooden case, one differential manometer for pressure 0 to 10 mm DWYER Instruments Inc, USA, was damaged, stainless steel surface plate on one side of tunnel was pressed.

The contents of the other 7 cases were delivered without any damage.

The Rota specialist repaired faults on the spot, while manometer and the connection plug must be replaced.

Kurt Randjelovic,
for Rota firm

J.Tserendendev,
Zonal Director

Prof.O.Scedrov,
CTA of UNIDO Project
DP/MON/82/002

Annex 5.14

Ulan Bator, 11 May 1987

P R O T O C O L

on the Installation of Rota Automatic Filling Line for Production of Sterile Products in Vials

The whole equipment was installed in the period from 3 May till 8 May 1987. Because of lack of correct technical documentation during preparatory work inside the building, provisional arrangements had to be done in installment of Pump-over agregate:

1) Connecting tubings between washing machine Jiffy I and Pump-over agregate had to be provisionally extended.

2) Connecting chute between washing machine Jiffy I and sterilizing tunnel ST-1 had to be extended as well, now without necessary covering shield.

The Rota firm shall send all necessary parts for definite connection of the said machines, the plexi shield over the extended chute, one prolonging stainless steel tubing and three teflon pipes for connecting of washing machine and pump as complete installation of the line.

Further, there shall be send all the parts damaged during transportation: one differential manometer for pressure 0 to 10 mm [illegible] Instruments Inc, USA, connecting plug for washing machine Jiffy I and one main switch on Laminar-flow which was damaged during the installation.

The printing plates for labels, which have some difference in hight of the types, shall be taken by Mr.Randjelovic to Rota firm, repaired and sent back to Ulan Bator in the period not longer than one month.

Rota firm shall send during one month period all necessary operating instructions for workers and for necessary repair operations.

Mongolian side expects that ten years delivery of spare parts will be provided by Rota firm.

The Rota firm is requested to send a spare parts catalogue with corresponding price list.

Because of short stay of Mr.Randjelovic, Rota specialist, in Ulan Bator only one day remained for necessary cooperation in putting into operation of the line and first trial production.

The Rota line was taken over by Mongolian side, the Experimental Centre of Applied Enzymology and Microbiology, on 11 May 1987, and the half year guarantee begins from May 1987.

Randjelovic

Kurt Randjelovic,
for Rota firm

J.Tserendendev
ECAEM Director

Prof.Oleg Feodrov
CTA of UNIDO
Project DP/MON/8
/002

Translation from Russian

WORK PLAN

of carrying out the sanitary-bacteriological examinations of premises, where the line for the sterile enzyme products production is installed.

Approved.
J.Tserendendev, Director of the Experimental Centre of Applied Enzymology and Microbiology
Prof. O.Ščedrov, Chief Technical Adviser of the Project DP/MON/82/002

	Description and scope of the work to be performed	To be done by	Name of the method and test	Culture media to be used	Duration of the test	Timing, from - to
1)	Determination of the air contamination by bacteria and counting of the microorganisms in 1 m^3 of the air.	O.Ščedrov, M.Čobanović, T.Adyasuren	Koch method	Meat-peptone agar, Saboure's dense medium	48 hours	From 4 May 1987, during the whole period of work of the sterile line.
2)	Determination of the presence of pathogenic microorganisms in the air: Staphylococcus aureus and Streptococcus haemolyticus.	"	"	Yolk-salt agar, blood medium	24 - 48 hours	"
3)	Microorganisms counting and exposure of pathogenic microorganisms in washing away of the workers hands.	"	The direct sowing method	Kissler's medium, meat--peptone agar, yolk-salt agar	48 hours	From 11 May 1987, during the whole period of work of the sterile line.
4)	Determination of the contamination by microorganisms on the workers clothes and other accessories and equipment in premises.	"	"	Kissler's medium, meat--peptone agar, 1% sugar broth	48 hours	"

1	2	3	4	5	6
5) Determination of pathogenic bacteria, Staphylococcus aureus and Streptococcus haemolyticus, in washing away of working clothes and equipment in premises.	O.Scedrov, M.Cobanović, T.Adyasuren	The direct sowing method	Endo agar, yolk-salt agar, blood medium	48 hours	From 13 May 1987, during the whole period of work of the sterile line.
6) Microorganisms counting in 1 ml of distilled water utilized for the glass containers washing.	"	"	Kissler's medium, meat-peptone agar.		From 11 May 1987, during the whole period of work of he sterile line.
7) The sterility determination of the prepared sterile distilled water.	"	"	Thioglycollate medium, Sabouraud liquide medium	48-72 hours, 10 days	"
8) The sterility determination of the sterilized glass containers and vials.	"	"	"	24-48 hours	"
9) Determination of the bacteria contamination of the Pancypsin solution before sterile filtration.	"	"	"	10 days	"
10) The sterility determination of the sterile Pancypsin solution after the filtration.	"	"	"	"	"
11) The sterility determination of the Pancypsin solution in vials before the freeze drying.	"	"	"	"	"

1	2	3	4	5	6
12) The sterility determination of the Pancypsin solution after the freeze drying.	O.Šćedrov, M.Čobanović, T.Adyasuren	The direct sowing method	Thioglycollate medium, Saboure's liquide medium	10 days	From 11 May 1987, during the whole period of work of the sterile line.
13) In the case of the presence of microorganisms in the sterile drug preparations, carrying out the tests of the presence of Esch. coli, Staph. aureus, Schigella and Salmonella groups.	"	"	Endo agar, Ploskirov agar, sodium salt medium, Bismuth sulfite agar, yolk-salt agar, blood agar.	48-72 hours	From 22 May 1987, during the whole period of work of the sterile line.

Prepared by: T.Adyasuren

M.Čobanović

29 April 1987

Professor Oleg Scedrov,
Chief Technical Adviser,
UNIDO Project DP/MON/82/002,
UNDP, Ulan Bator, Mongolia

15 May 1987

The Chief Technical Adviser's Remarks after Stay
in the "Rota" Line Sterile Premises

I entered into the sterile premises of the "Rota" filling line on 14 May 1987 and spent there about two hours, i.e. from 10.05 up to 11.50. I have observed the whole sterile work, the Pancypsin powder dissolving in the sterile distilled water, sterile filtration, the filling of vials on the "Rota" machine and capping with rubber stoppers, as well as collecting the filled vials into the plates. I was properly dressed, in accordance with the sterile conditions. The findings and recommendations are as follows:

- The air was bad inside of the entire sterile premises. An air conditioner is badly needed.
- The sterile coats without pockets are preferable.
- The dissolving of Pancypsin, performed in the sterile corridor (or sterile room No.2), has to be done in a glass container or flask, or in an enamel pot without damages, because the iron and other heavy metals from the damaged spots destroy enzymes, and in this case Pancypsin, and diminishes its activity.
- The table in the sterile room No. 2 has to be as simple as possible, without drawers and not covered by papers. If possible, a new painting is recommended.
- Sterile room No. 3 with the "Rota" filling machine. The pressure pump for Pancypsin solution sterile filtration was not sterile itself. It was located near (about half a meter) to the sterile filtered solution.
- The glass flask with the sterile Pancypsin solution has to be provided with two sterilized cotton stoppers. The second one is for closing of flasks after sterile filtration.
- To arrange a buffer bottle between the pump and the metal container with Pancypsin solution, would be avadvisable.
- If a pincers falls down, another is to be taken obligatory. Two sterile pincers are needed at least there.

- All the filled vials which were tumbled down, have to be removed.
- The number of chairs in the sterile room No. 3 have to be reduced to a smallerone.
- Consider whether the heating units (radiators) in all sterile rooms ar needed as they are dust collecting.
- Frequent washing of worker's hands (especially if in rubber gloves) by ethanol 96%, is not quite needed as the sterilisation is not achieved by ethanol.
- All movements in the sterile rooms have to be done slowly in order to minimise spinning of air.
- The atmosphere, the air, in the sterile rooms was bad, as the chlorine is nipping the breathing organs. The exchange of air is necessary, therefore pouring of the fresh air from outside using air conditioners with sterile filters is required.
- The rule not to talk in the sterile premises was observed very well. This is very good and should be further adhered to.
- The freeze driers filling was done by a worker properly dressed, but without rubber gloves. His hands were in close contact with the filled vials semi capped with the rubber stoppers. He has to wear rubber gloves.

(Professor Oleg Scedrov)

Translation from Russian

WORK PLAN

of carrying out the pharmacological examinations of sterile enzyme preparations and pyrogen free water.

Approved.
J. Tserendendev, Director of the Experimental Centre of Applied Enzymology and Microbiology.
Prof. O. Šćedrov, Chief Technical Adviser of the Project DP/MON/82/002.

6 May 1987

Description and scope of the work to be performed	To be done by	Name of the method and test	Animals to be used	Duration of the test	Timing, from - to
1) The pyrogen test of the prepared distilled water	O.Šćedrov, M.Ćobanović, K.Kabden	FIP, p.155	Rabbits	48 hours	From 11 May 1987, during the whole period of work of the sterile line.
2) The pyrogen test of Pancypsin in vials after freeze drying	"	FIP, p.155; USP XXI, p.1181	Rabbits	48 hours	"
3) The toxicity test of Pancypsin in vials after freeze drying	"	FIP, p.154; USP XXI, p.1198	Mice	72 hours	"

Prepared by: M.Ćobanović
K.Kabden

Annex 5.18.1

Pancypsin Production Technology

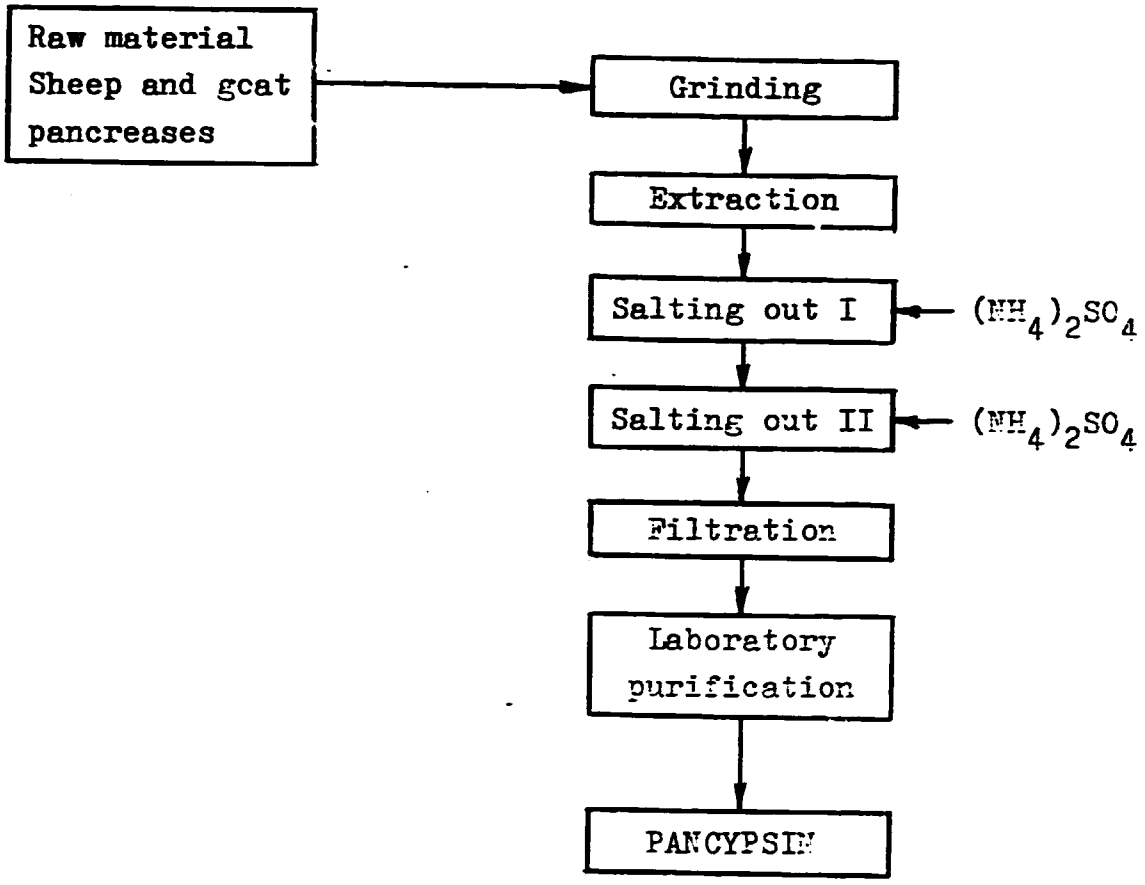
The frozen pancreas is ground in a meat grinder and then extracted with water acidified with sulphuric acid in an enamelled kettle. After extraction the mixture is sifted through a stainless steel sieve. The extract is colected in a reservoir and first the impurities, then the product, are salted out by means of ammonium sulphate. The precipitate of crude proenzymes is collected by vacuum filtration on stainless steel filters and the obtained product is then purified on the laboratory scale, using activation of the proenzymes to enzymes and repeated salting out of the pure product. After dialyse the product is freeze dried.

From 100 kg of pancreas, approximately 250 g of Pancypsin is obtained. The processing of 100 kg of pancreas per day will yield approximately 75 kg of Pancypsin per year.

The production equipment is expected to be used for the processing of chymotrypsin and trypsin, as well. The technologies for all these substances are similar in the first stage of the production.

Annex 5.18.2

PRODUCTION OF PANCYPSIN (SCHEME)



Annex 5.19.1

Dry Bile Production Technology

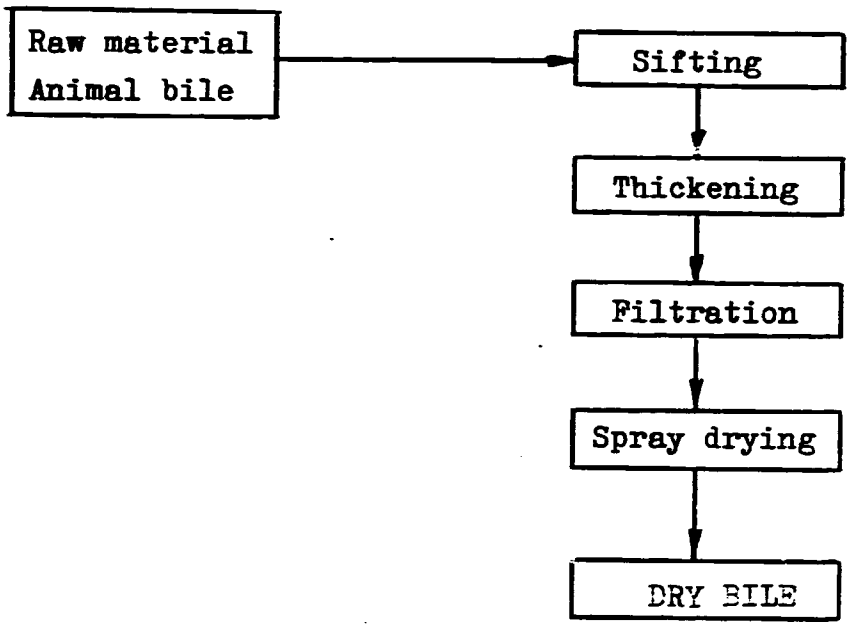
The collected animal bile is first evaporated to about 1/5 of its original volume on the film evaporator Anhydro. The obtained thickened product is then filtered and dried on the spray-dryer Anhydro. In case of higher collection capacity, it can be stored before drying in frozen state for future processing.

The production of dry bile in ECAEM is fitted with the equipment, the capacity of which amounts to 13 tons of dry product per year, running in two shifts during the whole year.

This equipment could be as well utilized in the production of peptone.

Annex 5.19.2

PRODUCTION OF DRY BILE (SCHEME)



Annex 5.20.1

Chymotrypsin and Trypsin
Production Technology

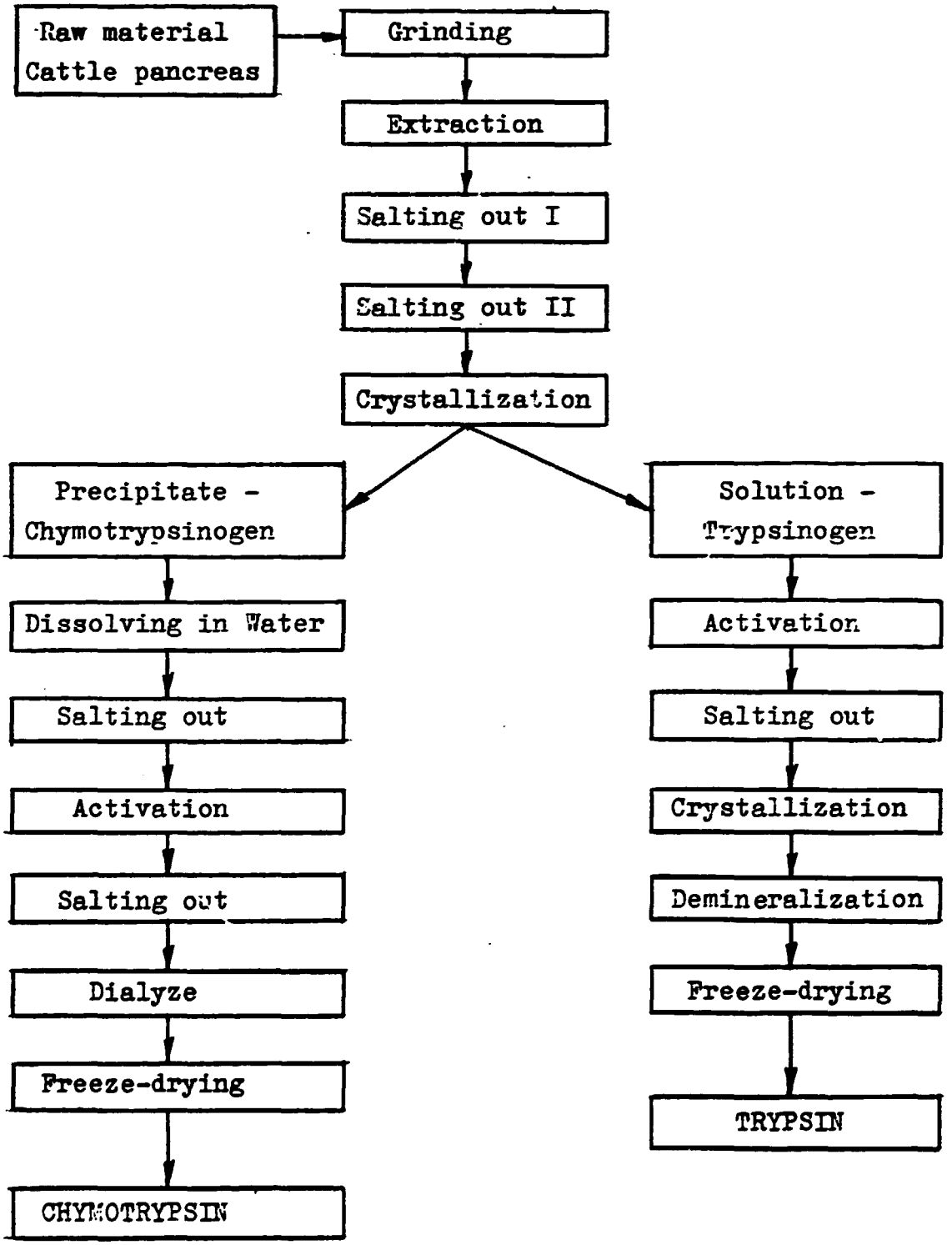
The first steps of the production technology is similar to the processing of Pancypsin. The precipitate of crude proenzymes after filtration is purified as well on the laboratory scale. It is dissolved in water and from the solution first the crystals of chymotrypsinogen are obtained through crystallization. The crystals are then collected by filtration, the filtrate being collected for future elaborating of trypsin. The crystals of chymotrypsinogen are dissolved in water and the proenzyme is activated into enzyme, salted out from the solution, once again purified, and finally dialyzed and freeze dried.

The solution of trypsinogen is first processed as well by activation, the product is then salted out from the solution, collected and, after dissolving in buffer, the enzyme is crystallized.

From 100 kg of cattle pancreas expected is approximately 70 g of chymotrypsin and 50 g of trypsin, both in form of freeze dried powder.

Annex 5.20.2

PRODUCTION OF CHYMOTRYPSIN AND TRYPSIN (SCHEME)



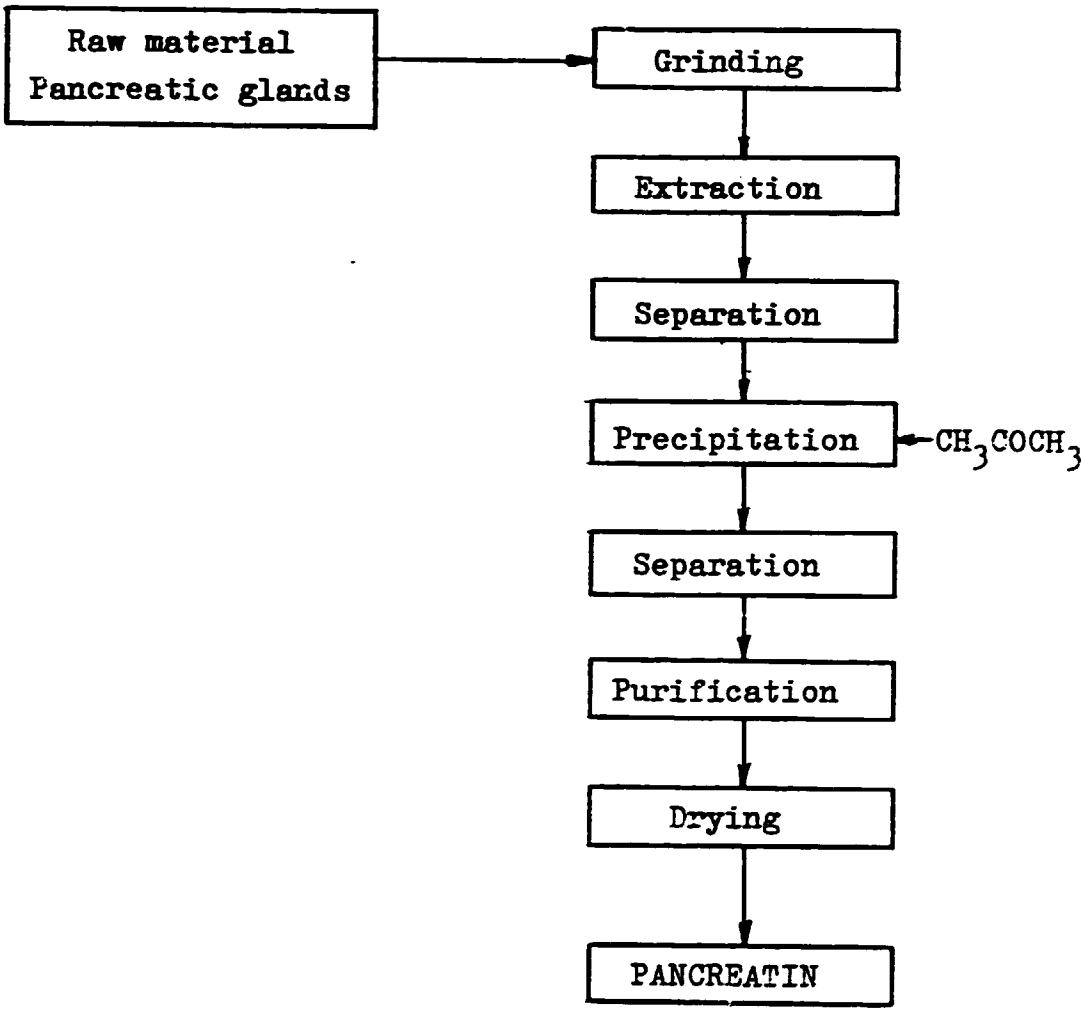
Annex 5.21.1

Pancreatin Production Technology

According to the prepared technology the ground, activated pancreas is transferred into a kettle where it is extracted with water for 3 hours, in presence of the necessary ingredients. After extraction the mixture is sifted, the filtrate is collected in a reservoir and the active substance is precipitated with acetone. After filtration and further purification, the product is finally dried in a dryer. From 100 kg of pancreas approximately 10 kg of pancreatin is obtained.

Annex 5.21.2

PRODUCTION OF PANCREATIN (SCHEME)



Annex 5.22.1

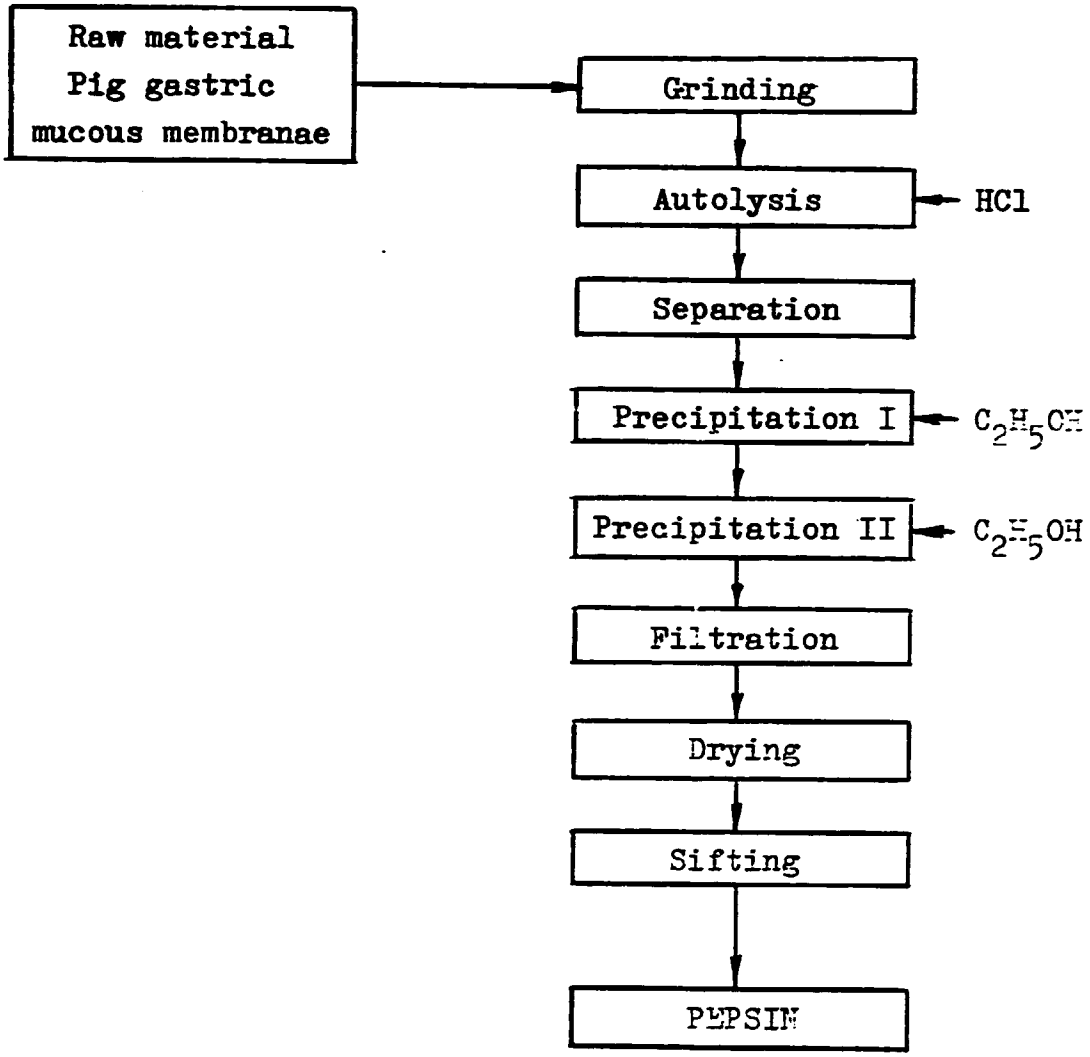
Medical Grade Pepsin
Production Technology

According to the prepared technology the pig gastric mucose is ground into an enamelled kettle and hydrochloric acid is added. The mixture is then autolysed at a temperature of 40°C. The autolysate obtained is filtered through a sieve into a reservoir, in which is purified and precipitated. The product is collected by filtration using a vacuum filter, and dried in a dryer.

From 100 kg of the raw material approximately 1.8 kg of pepsin is expected.

Annex 5.22.2

PRODUCTION OF PEPSIN (SCHEME)



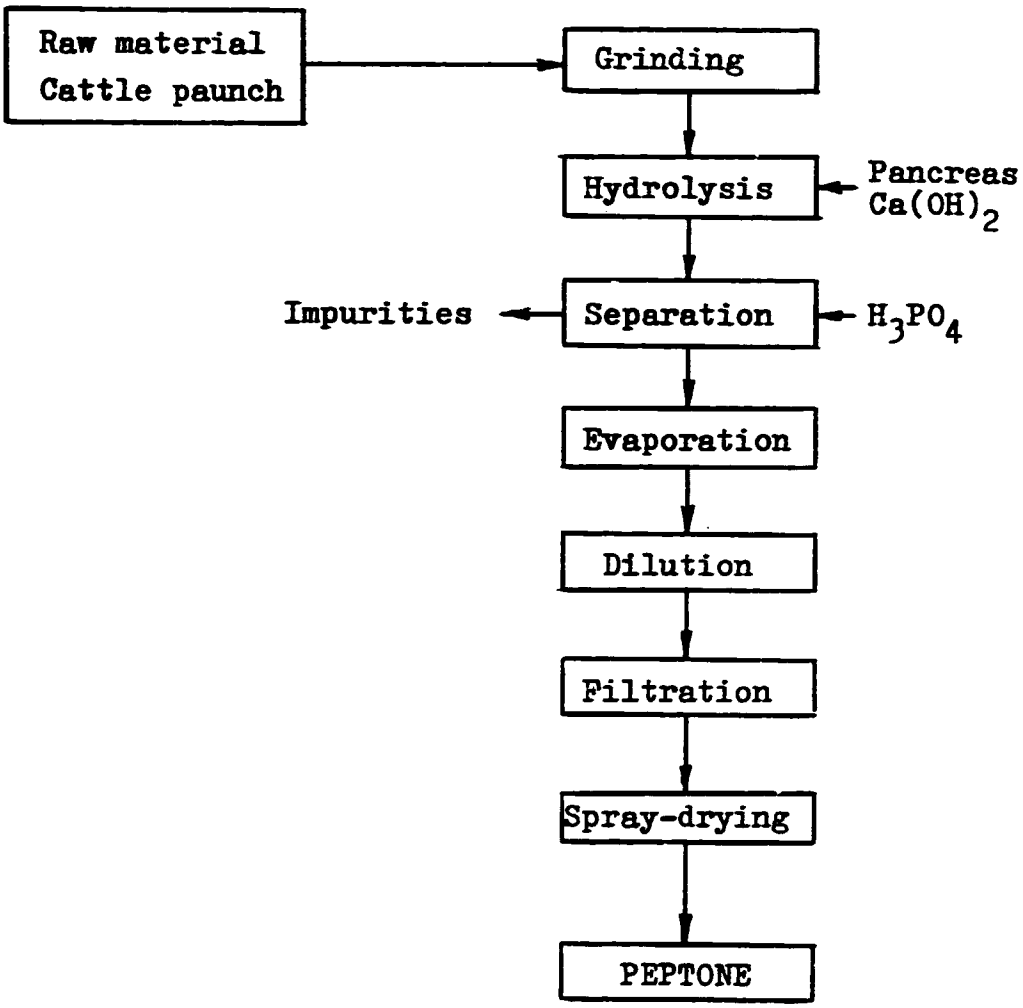
Annex 5.23.1

Peptone Production Technology

The raw material (cattle paunch) is ground in a meat grinder and transferred into a jacketed enamelled kettle, where it is mixed with water. The mixture is then boiled and after cooling to 45°C, previously activated ground pancreas is added. The hydrolysis of proteins is then carried out for 10 hours at a constant temperature of 45°C and pH 8 to 8.5, the pH being adjusted from time to time with calcium hydroxide. After hydrolysis the mixture is adjusted with phosphoric acid, filtered or clarified in a separator and collected. The solution of peptone is then thickened to about 1/5 of the original volume. The product is filtered and finally dried in a spray-dryer. From 300 kg of the raw material approximately 17.5 kg of peptone is expected.

Annex 5.23.2

PRODUCTION OF PEPTONE (SCHEME)



Annex 5.24

CTA's Comments on Mr. V. A. Tumanyan's Report "Assistance to the Dairy and Cheese Production by Improvement of the Raw Milk Tests and the Quality Control", SI/MON/85/802, January 1987, pp. 73.

First part of the Report deals with the problems of milk collecting and distribution in Mongolia, particularly in Ulan Bator. There is a problem of microbial contamination of the milk. The second part of the Report deals with cheese making, and only the brine cheese was produced experimentaly using rennet for clotting of the milk. The origin, quality and activity of the rennet was not mentioned in the Report. - Later on (28 April 1987) Dr. Gombo (5.3.9) informed the CTA that Mr. Tumanyan's rennet was a calf rennet with an approximately milk clotting activity of 1 : 100 000. - On the p.36 of the Report it was quoted that 2.5 g of the rennet was used for clotting of 100 kg of milk. In Ulan Bator 34 000 tons of milk were consumed in 1986 (the Report p. 25). According to the CTA, maybe a third of it could be used for cheese manufacture, what means about 10 000 tons of milk could be clotted with 250 kg of rennet. More or less the same amount of pepsin will be needed for cheese making. As 36 kg of brine cheese was manufactured from 100 kg of milk (Mr. Tumanyan's Report p. 27), consequently from 10 000 tons of milk 3 600 tons of brine cheese could be obtained. The question is the amount of cheese consumed yearly in Mongolia.

Annex 5.25 Translation from Russian

Professor Oleg Ščedrov Ulan Bator, 20 April 1987

Processing of Blood

The blood utilization is often a slaughterhouse problem.
If disregarded, the blood causes environmental pollution.
The easiest way of utilization is to sterilize the whole blood under pressure of 3 atm at 180°C during 3 hours, and evaporate the water after. The blood meal obtained has 12% of moisture, and is used for animal feed or fertilizer. During such processing the most valuable ingredients of blood are destroyed.
Better and more up-to-date procedure is spray-drying the blood to dry plasma and haemoglobin.
For a viable production about 2 000 tons of blood per annum is needed. As 10 kg of blood can be collected from a bovine, it will amount to about 200 000 cattle per year. Depending on work days per year, it will be 700 to 1 200 cattle per day, or 7 to 12 tons of blood per day.
Spray-drying of blood is not too complicated a procedure. One must take measures against contamination by microorganisms. After collecting the blood of animals freshly killed with a hollow knife, an anticoagulant is added. Separation in a special type centrifuge for blood is easy and effective. Maybe a centrifuge unit (or separator for blood) from the firm of "Alfa Laval" or "Westfalia" might be used. After treatment of the erythrocytes in homogenizer to extract haemoglobin, and a separate evaporation of plasma and haemoglobin (the "Alfa Laval" Centri-Therm Evapora CT-6 is very effective and advisable), the two products have to be spray-dried separately (using maybe "Niro Atomizer" machine).
The general review of the sequence of the production operations is given on page 116 (Annex 5.27.1).
The most important equipment items are:
- Centrifuge unit (or separator) for blood,
- Homogenizer for erythrocytes,
- Evaporator for sensitive substances,
- Spray-dryer.

Such a plant has to be built close by a slaughterhouse, as any transportation of blood is difficult and has no economical reason, and also because of the infrastructure needed (steam, cooling facilities, water supply, electricity, etc.).
The expected amount of the entire investment (including production equipment, installation, electrical works, construction of the building, instrumentation, and laboratory equipment and apparatus) for said processing of 2 000 to 3 000 tons of blood per annum would be total approximately (in 1983):

$ 2 000 000.-

More precise data and complete know-how can be obtained from the firm of "Niro Atomizer", Soeborg, Copenhagen, Denmark, or "Phylaxia", Budapest, Hungary.
For the above mentioned production capacity in three shifts and five workdays a week, the following personnel would be needed:
- One biochemical technologist or veterinarian,
- Three technicians,
- Six skilled workers.

Total: Ten persons directly engaged in the production.
From 2 000 tons of blood per annum one can obtain:
- Dry plasma, with 5% of moisture, 100 tons,
- Dry haemoglobin, with 5% of moisture, 260 tons.

As the price (in 1983) of dry plasma was $ 3.00 per kg, it will amount to $ 300 000.- per annum.
The dry haemoglobin costs $ 1.00 per kg, and for annual production it will be $ 260 000.- The total amount will be $ 560 000.- per year.
Dry plasma is widely used in food processing industry, for soups, canned products, as additional protein, and also as an emulsifier and a stabilizer.
Dry haemoglobin serves for animal feed, with much higher nutritional value than blood meal, and a lesser quantity is used for sausages production.
This represents only the first step of production. Futher processing of plasma includes defibrination with more anticoagulants and Ca-salts and the second centrifugation.

After spray-drying, pure 98% albumin is obtained. For this production a further investment of about

$ 500 000.-

was required (in 1983).

So the total sum of investment would be about:

$ 2 500 000.- (in 1983).

From dry plasma 98% albumin with 40% yield (or 2% of whole blood) can be extracted. The capacity of production considered as 100 tons of dry plasma per year, yields 40 tons of 98% albumin. The price of this albumin amounts to about $ 30.- per kg, and the annual production would cost (in 1983):

$ 1 200 000.-

Albumin is widely used for cosmetics, and also serves as medical a diagnostic and special quality control reagent.

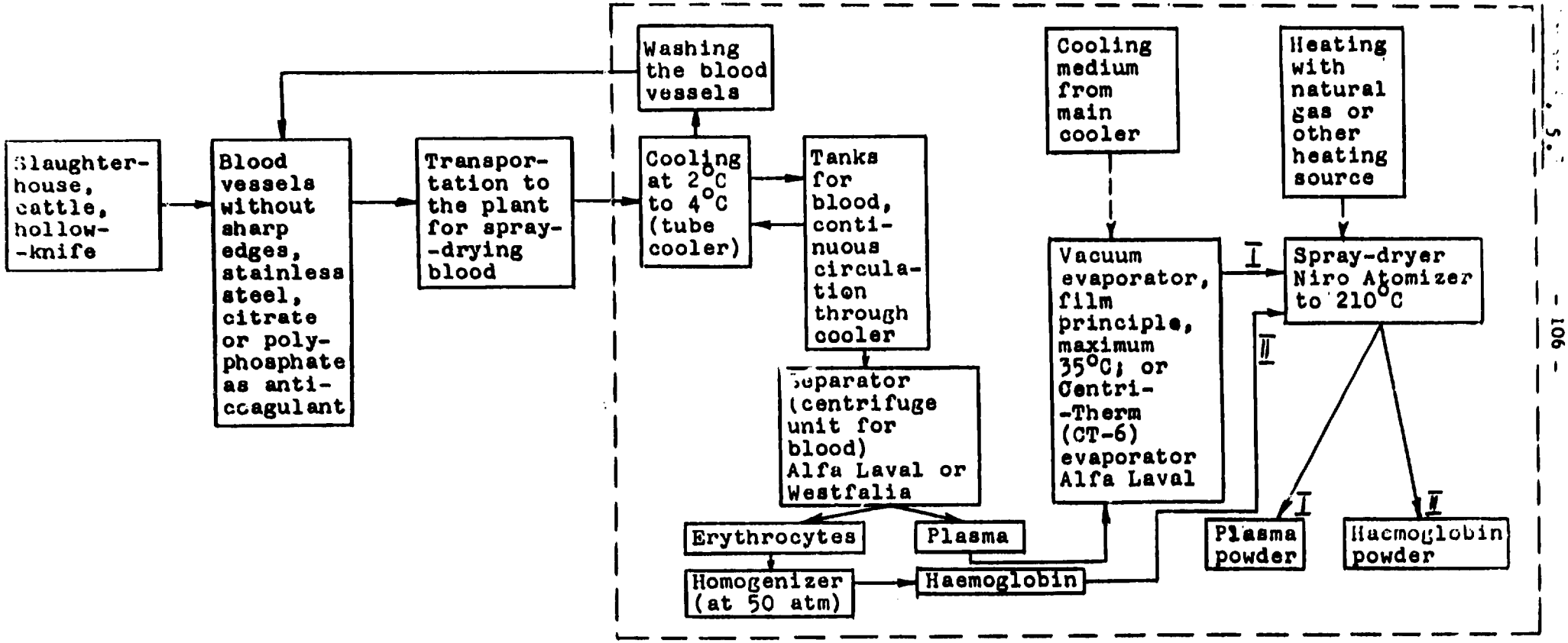
Further purification of plasma enables the isolation of high purity albumin, fibrin, and immunoglobulins for the veterinarian medicine as well. The procedure are not easy and require costly equipment.

On the other hand, the spray-dried haemoglobin can be hydrolysed into many valuable amino acids by a fairly easy and not too expensive technology (for instance according to C. C. Hellquist, Ellco Protein A B, B. R. Deutschland Offenlegungsschrift 2 703 742, 18 Aug. 1977, or O. Ščedrov et al., Acta Med. Saliniana 1983, 12, No. 1, 5-16). Amino acids obtained can be used as additional nutritives in human diseases accompanied by a loss of body proteins, and in foodstuff industry, especially as meat extracts.

Small slaughterhouses in general disregarded the blood. There is an easy way of preparing blood as a special additive to poultry and pig feed. Only a refrigerator unit at +4°C and a common meat grinder are needed.

Spray-dried blood production, general review

(7 000 litres of blood per day)



One batch processing lasts two shifts from 6 a.m. to 10 p.m.
Night shift washes and disinfects the whole equipment of the plant.

Annex 5.6

Milka Čonanović
Quality Control Expert
UNDP, Ulan Bator, Mongolia

23 March 1987

Evaluation of the Quality and Specification of the Existing Raw Materials, Semi-products and Final Products at the Experimental Centre of Applied Enzymology and Microbiology in Ulan Bator

The director of the Experimental Centre of Applied Enzymology and Microbiology (ECAEM), as our counterpart, and some of his co-workers informed us of the existing situation of the quality of the products produced in the Centre.

The regular quality control tasks are carried out only in connection with the final products such as: Pancypsin, Trypsin, Chymotrypsin and Dry Bile. Therefore, the quality of the raw materials used for the production of pharmaceutical products is not carried out.

However, all material entering into the process for manufacture of drugs is generally considered as raw material, and as such has to be obligatorily controlled. Consequently, security of people's health depends on control of the raw material used for production of drugs. It must be assumed that all delivered containers of raw materials contain the corresponding declared products.

With exception of Pancypsin, all other mentioned enzume products appear to be only controlled from time to time by some physically-chemical methods.

The testing methods used in ECAEM for quality control of mentioned enzymes cannot be found in new pharmacopoeias and other corresponding regulations. One may say that after a rapid growth of new exact testing methods, twenty years ago, the pharmaceutical industry made a gigantic step forward in respect of improvement of quality control of the pharmaceutical products. Both, control of production and of analytical methods are considerable. Such a gigantic improvement is due to new precise apparatuses and improved condition of operations which enable increased safety and quick detection of possible or eventual errors.

On the basis of the current status of the quality control of enzyme products in the Centre, as well as on the basis of the cooperation with the counterparts, one may say that the quality control is in its very initial stage and practically started with arrival of the team of the project experts in February 1987.

There are numerous problems arising in the course of analyses, such as:

- The quality control is carried out only by three specialists and one laboratory worker.

-The working area is smaller than 10 square meters, which is inadequate for performance of various operations and preparation of reagents for analyses, titration, washing and drying of the laboratory glasswares.

- Washing and drying of the laboratory glasswares take place in the very small passing corridor. The working conditions are unsatisfactory.

- The balance unit is located very far from the quality control laboratory so that the employees have to go through the corridors and other premises in order to determine weight of the samples, which also increases possible risk of errors and air or mechanical contamination and diminished the authenticity and reliability of analytical results.

- To obtain reliable and exact results from assays, some special precautions should be taken and an appropriate working discipline introduced.

- Corresponding glassware has to be provided for the quality control section. The glassware must be carefully cleaned and defatted. Protein and other surface active materials tend to be absorbed on by glass walls and may not be removed by ordinary rinsing with distilled water. Glassware which was in contact with a protein solution should be treated with the solution of potassium dichromate in sulfuric acid, rinsed with hot water, then with distilled water and well dried.

- The highest grade of purity of used reagents is required. They should be quality "Pro Analysis". "Purum" quality ought not be used. Special attention should be paid to occurence of traces

of heavy metals or other specific chemical impurities which might interfere with enzymes.

- There is no control of water for laboratory use. Such a control is needed in order to to ascertain whether the quality of water needed for carrying out analyses has been reached. We have to say that the quality of water is vary important for correct analytical tests.

- Enzyme activity is determined by the quantity of substrate transformed, or product formed, per time unit. The reaction measurements depends on the experimental conditions such as: temperature, pH, ionic strength of substrate, and presence or absence of inhibitors and activators. Only under the mentioned conditions in the assay procedure, the enzyme activity can be defined. Whenever any condition is changed, the kinetic measurements of the activity cannot have a well defined relationship to the units of the enzyme preparation.

- The substrate used must have a relevant specificity to the reaction catalysed. The highest substrate purity should be required for a quantative assay of enzyme activity, as well as precise control of measured data.

- It is very important for the activity of the unknown enzyme preparation the use of reference standards with known activity. It is therefore necessary for ECAEM to order all the standards and to prepare the national standards or working standards.

- The ionization of active groups in substarte from binding centre usualy depends on pH and influence the catalytical activity of the relevant enzyme. Therefore, the exact pH checking by potentiometer is obligatory in all enzyme assays. The pH determination by pH-papers can never achieve the accurancy required.

- The equipment should be chosen so, that the optimal sensitivity and accurary can be reached. It has to be installed by a specialist and it should be regularly inspected by a component agent or institution.

(Milka Cobanovic)

co.-Mr.J.Litoukhin,
Resident Representative,
UNDP,Ulan Bator,Mongolia
-Dr. Z.Csizèr,UNIDO,Vienna

Annex 5.27

Short Description of the Total Proteolytic Activity Determination by the Armour Method

The total proteolytic activity can be determined by haemoglobin as a substrate. Trichloroacetic acid filtrates of the digested haemoglobin are treated by Folin-Ciocalteu phenol reagent. The blue colour developed due to tyrosine is measured colorimetrically. The activity should be expressed as Armour units in relationship to the activity of a reference (or a working) standard of crystalline trypsin.

The United States Pharmacopeia

TWENTY-FIRST REVISION

Official from January 1, 1985

The National Formulary

SIXTEENTH EDITION

Official from January 1, 1985

United States Pharmacopeial Convention, Inc.
12601 Twinbrook Parkway, Rockville, Md. 20852

Packaging and storage— Preserve in single-dose or in multiple-dose containers, preferably of Type I or Type II glass.

Labeling— Label the Injection to indicate that it is to be diluted to the appropriate strength with Sterile Water for Injection or other suitable fluid prior to administration.

Identification— The *Assay preparation*, prepared as directed in the *Assay*, exhibits an absorption maximum at about 360 nm when tested as directed for *Procedure* in the *Assay*.

Pyrogen— When diluted with Sodium Chloride Injection to contain 0.1 µg of chromium per mL, it meets the requirements of the *Pyrogen Test* (151).

pH (791): between 1.5 and 2.5.

Other requirements— It meets the requirements under *Injections* (1).

Assay—

Sodium chloride solution— Dissolve 10.8 g of sodium chloride in water, dilute with water to 2000 mL, and mix.

Chromium stock solution— Transfer 2.829 g of potassium dichromate, accurately weighed, to a 1000-mL volumetric flask, dissolve in water, dilute with water to volume, and mix. This solution contains 1000 µg of chromium per mL. Store in a polyethylene bottle.

Standard preparations— Pipet 10 mL of the *Chromium stock solution* into a 1000-mL volumetric flask, dilute with *Sodium chloride solution* to volume, and mix. Transfer 10.0 mL and 20.0 mL, respectively, of this solution to separate 100-mL volumetric flasks, and transfer 15.0 mL and 20.0 mL, respectively, of the solution to separate 50-mL volumetric flasks. Dilute the contents of each flask with *Sodium chloride solution* to volume, and mix. These *Standard preparations* contain, respectively, 1.0, 2.0, 3.0, and 4.0 µg of chromium per mL.

Assay preparation— Transfer an accurately measured volume of Chromic Chloride Injection, equivalent to about 60 µg of chromium, to a 25-mL volumetric flask, dilute with water to volume, and mix.

Procedure— Concomitantly determine the absorbances of the *Standard preparations* and the *Assay preparation* at the chromium emission line of 357.9 nm, with a suitable atomic absorption spectrophotometer (see *Spectrophotometry and Light-scattering* (851)) equipped with a chromium hollow-cathode lamp and an air-acetylene flame, using the *Sodium chloride solution* as the blank. Plot the absorbances of the *Standard preparations* versus concentration, in µg per mL, of chromium, and draw the straight line best fitting the four plotted points. From the graph so obtained, determine the concentration, in µg per mL, of chromium in the *Assay preparation*. Calculate the quantity, in µg, of chromium in each mL of the Injection taken by the formula $25C/V$, in which C is the concentration, in µg per mL, of chromium in the *Assay preparation*, and V is the volume, in mL, of Injection taken.

Chromic Phosphate P32 Suspension—*see* Phosphate P32 Suspension, Chromic

Sodium Chromate Cr 51 Injection

Chromic acid ($H_2{}^{51}CrO_4$), disodium salt.
Disodium chromate ($Na_2{}^{51}CrO_4$) [7775-11-3].

» Sodium Chromate Cr 51 Injection is a sterile solution of radioactive chromium (^{51}Cr) processed in the form of sodium chromate in Water for Injection. For those uses where an isotonic solution is required, Sodium Chloride may be added in appropriate amounts as provided under *Injections* (1). Chromium 51 is produced by the neutron bombardment of enriched chromium 50.

Sodium Chromate Cr 51 Injection contains not less than 90.0 percent and not more than 110.0 percent of the labeled amount of ^{51}Cr as sodium chromate expressed in millicuries per mL at the time indicated in the labeling. The sodium chromate content is not less than 90.0 percent and not more than 110.0 percent of the labeled amount. The specific activity is not less than 10 millicuries per mg of sodium chromate at the end of the expiry period. Other chemical forms of radioactivity do not exceed 10.0 percent of the total radioactivity.

Packaging and storage— Preserve in single-dose or in multiple-dose containers.

Labeling— Label it to include the following, in addition to the information specified for *Labeling* under *Injections* (1): the time and date of calibration; the amount of sodium chromate expressed in µg per mL; the amount of ^{51}Cr as sodium chromate expressed as total millicuries and as millicuries per mL at the time of calibration; a statement to indicate whether the contents are intended for diagnostic or therapeutic use; the expiration date; and the statement, "Caution—Radioactive Material." The labeling indicates that in making dosage calculations, correction is to be made for radioactive decay and the quantity of chromium, and also indicates that the radioactive half-life of ^{51}Cr is 27.8 days.

Reference standard— *USP Endotoxin Reference Standard*.

Radionuclide identification (see *Radioactivity* (821))— Its gamma-ray spectrum is identical to that of a specimen of ^{51}Cr of known purity that exhibits a photopeak having an energy of 0.320 MeV.

Bacterial endotoxins— It meets the requirements of the *Bacterial Endotoxins Test* (85), the limit of endotoxin content being not more than $175/V$ USP Endotoxin Unit per mL of the Injection, when compared with the USP Endotoxin RS, in which V is the maximum recommended total dose, in mL, at the expiration date or time.

pH (791): between 7.5 and 8.5.

Radiochemical purity— Place a volume of Injection, appropriately diluted such that it provides a count rate of about 20,000 counts per minute, about 25 mm from one end of a 25- × 300-mm strip of chromatographic paper (see *Chromatography* (621)), and immediately develop with a mixture of 5 parts of water, 2 parts of dilute alcohol (9.5 in 10), and 1 part of ammonium hydroxide. Dry the chromatogram in air, and determine the radioactivity distribution by scanning the chromatogram with a suitable collimated radiation detector. The radioactivity of the chromate band is not less than 90.0% of the total radioactivity. The R_f value for the chromate band falls within ±10% of the value found for a known sodium chromate specimen when determined under identical conditions.

Other requirements— It meets the requirements under *Injections* (1), except that it is not subject to the recommendation on *Volume in Container*.

Assay for sodium chromate— Prepare a Standard solution of sodium chromate adjusted with sodium bicarbonate solution (1 in 100) to a pH of 8.0 ± 0.5 and containing 1.4 µg of sodium chromate per mL. Determine the absorbances of the Standard solution and of Sodium Chromate Cr 51 Injection, respectively, in 5-cm cells at the wavelength of maximum absorbance at about 370 nm, with a suitable spectrophotometer, using water as the blank. If the absorbance of the Injection is not within 10% of that of the Standard solution, appropriately dilute either the Injection or the Standard solution. If the Injection is diluted, calculate the quantity, in µg, of Na_2CrO_4 per mL of the Injection taken by the formula $1.4D_t(A_t/A_S)$, in which D_U is the dilution factor for the Injection and A_t and A_S are the absorbances of the Injection and the Standard solution, respectively. If the Standard solution is diluted, use $1/D_S$ in which D_S is the dilution factor for the Standard, in place of D_t.

Assay for radioactivity— Using a suitable counting assembly (see *Selection of a Counting Assembly* under *Radioactivity* (821)), determine the radioactivity, in µCi per mL, of Sodium Chromate Cr 51 Injection by use of a calibrated system as directed under *Radioactivity* (821).

Chymotrypsin

Chymotrypsin.
Chymotrypsin [9004-07-3].

» Chymotrypsin is a proteolytic enzyme crystallized

from an extract of the pancreas gland of the ox, *Bos taurus* Linné (Fam. Bovidae). It contains not less than 1000 USP Chymotrypsin Units in each mg, calculated on the dried basis, and not less than 90.0 percent and not more than 110.0 percent of the labeled potency, as determined by the *Assay*.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

Reference standards—*USP Chymotrypsin Reference Standard*—Keep container tightly closed and store in a refrigerator. Allow contents to reach room temperature before opening, and do not dry before using. *USP Trypsin Crystallized Reference Standard*—Keep container tightly closed and store in a refrigerator. Allow contents to reach room temperature before opening, and do not dry before using.

Microbial limits—It meets the requirements of the tests for absence of *Pseudomonas aeruginosa* and *Salmonella* species and *Staphylococcus aureus* under *Microbial Limit Tests* (61).

Loss on drying (731)—Dry it in a vacuum oven at 60° for 4 hours: it loses not more than 5.0% of its weight.

Residue on ignition (281): not more than 2.5%.

Trypsin—

Chymotrypsin solution—Dissolve 100 mg in 10.0 mL of water.

pH 8.1 Tris(hydroxymethyl)aminomethane buffer, 0.08 M—Dissolve 294 mg of calcium chloride in 40 mL of 0.20 *M* tris(hydroxymethyl)aminomethane, adjust with 1 *N* hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

Substrate solution—Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *pH 8.1 Tris(hydroxymethyl)aminomethane buffer, 0.08 M*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

Procedure—[NOTE—Determine the suitability of the substrate by performing the *Procedure* using the appropriate amount of USP Trypsin Crystallized RS in place of the test specimen.] By means of a micro pipet, transfer 50 µL of *Chymotrypsin solution* to a depression on a white spot plate. Add 0.2 mL of *Substrate solution*: no purple color develops within 3 minutes (not more than 1% of trypsin).

Assay—

pH 7.0 phosphate buffer, fifteenth-molar—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of dibasic sodium phosphate solution. If necessary, adjust to a pH of 7.0 by the dropwise addition of dibasic sodium phosphate solution.

Substrate solution—Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying Chymotrypsin, in about 50 mL of *pH 7.0 phosphate buffer, fifteenth-molar*, with warming. When the solution is cool, dilute with additional pH 7.0 buffer to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

Chymotrypsin solution—Dissolve a sufficient quantity of Chymotrypsin, accurately weighed, in 0.0012 *N* hydrochloric acid to yield a solution containing between 12 and 16 USP Chymotrypsin Units per mL. The dilution is correct if, during the conduct of the assay, there is a change in absorbance of between 0.008 and 0.012 in each 30-second interval.

Procedure—[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Procedure* using USP Chymotrypsin RS in place of the assay specimen.] Conduct the assay in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 0.1° in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance in order to assure that the temperature does not change by more than 0.5°. Pipet 0.2 mL of 0.0012 *N* hydrochloric acid and 3.0 mL of *Substrate solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.200 at 237 nm. Pipet 0.2 mL of *Chymotrypsin solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for not less than 3 minutes, repeat the test and, if necessary, use a lower concentration. The duplicate determination at the same dilution matches the first determination in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this assay. Calculate the number of USP Chymotrypsin Units per mg by the formula $(A_2 - A_1)/(0.0075TW)$, in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Chymotrypsin in the volume of solution used in determining the absorbance.

Chymotrypsin for Ophthalmic Solution

» Chymotrypsin for Ophthalmic Solution is sterile Chymotrypsin. When constituted as directed in the labeling, it yields a solution containing not less than 80.0 percent and not more than 120.0 percent of the labeled potency.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.

Completeness of solution (641)—It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

Identification—Prepare a *Substrate solution* as follows. Transfer 237.0 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, to a 100-mL volumetric flask, add 2 mL of alcohol, and swirl until solution is effected. Add 20 mL of *pH 7.0 phosphate buffer, fifteenth-molar*, prepared as directed in the *Assay* under *Chymotrypsin*, add 1 mL of methyl red–methylene blue TS, and dilute with water to volume. If necessary, adjust to a pH of 7.0 by the dropwise addition of monobasic potassium phosphate solution, prepared by dissolving 4.54 g of monobasic potassium phosphate in sufficient water to yield 500 mL of solution. Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 1 mL of saline TS, transfer 0.2 mL to a suitable dish, and add 0.2 mL of *Substrate solution*: a purple color is produced within 3 minutes (*distinction from trypsin, which produces no purple color within 3 minutes*).

pH (791): between 4.3 and 8.7, in the solution constituted as directed in the labeling.

Other requirements—It meets the requirements of the test for *Trypsin* under *Chymotrypsin*. It meets also the requirements for *Sterility Tests* (71) and for *Uniformity of Dosage Units* (905).

Assay—Proceed with Chymotrypsin for Ophthalmic Solution as directed in the *Assay* under *Chymotrypsin*, but use the following as the *Chymotrypsin solution*: Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 5.0 mL of 0.0012 *N* hydrochloric acid. Dilute an accurately measured volume (V, in mL) of this solution, equivalent to about 300 USP Chymotrypsin Units, with 0.0012 *N* hydrochloric acid to 25.0 mL. Calculate the number of USP Chymotrypsin Units per vial by the formula $300(5/V)(A_2 - A_1)/[T(2.4)(0.0075)]$, in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time in minutes between the initial and final readings, and 2.4 is the number of USP Chymotrypsin Units in the solution on which the absorbance was determined.

Tropicamide Ophthalmic Solution

» Tropicamide Ophthalmic Solution is a sterile, aqueous solution of Tropicamide. It contains not less than 95.0 percent and not more than 105.0 percent of the labeled amount of $C_{17}H_{20}N_2O_2$. It contains a suitable antimicrobial agent, and may contain suitable substances to increase its viscosity.

Packaging and storage—Preserve in tight containers, and avoid freezing.

Reference standard—*USP Tropicamide Reference Standard*—Dry in vacuum over phosphorus pentoxide at 80° for 4 hours before using.

Identification—

A: Extract 10 mL of it with 25 mL of chloroform, filter the chloroform extract through dry, folded filter paper, and evaporate the filtrate to dryness: the residue so obtained responds to *Identification test A* under *Tropicamide*.

B: The ultraviolet absorption spectrum of the solution employed for measurement of absorbance in the *Assay* exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Tropicamide RS, concomitantly measured.

Sterility—It meets the requirements under *Sterility Tests* ⟨71⟩.

pH ⟨791⟩: between 4.0 and 5.8.

Assay—Transfer an accurately measured volume of Tropicamide Ophthalmic Solution, equivalent to about 30 mg of tropicamide, to a 100-mL volumetric flask, add water to volume, and mix. Transfer 10.0 mL of this solution to a separator, add 2 mL of sodium carbonate solution (1 in 10), extract with four 20-mL portions of chloroform, and combine the extracts in a second separator. Wash the combined extracts with a 25-mL portion of pH 6.5 phosphate buffer (see *Buffer Solutions* in the section, *Reagents, Indicators, and Solutions*), and transfer to another separator. Wash the aqueous layer with 10 mL of chloroform, and add it to the extracts. Extract the chloroform solution with four 20-mL portions of dilute sulfuric acid (1 in 6), combine the acid extracts in a 100-mL volumetric flask, and add the dilute acid to volume. Dissolve an accurately weighed quantity of USP Tropicamide RS in dilute sulfuric acid (1 in 6), and dilute quantitatively and stepwise with the same solvent to obtain a Standard solution having a known concentration of about 30 μg per mL. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorbance at about 253 nm, with a suitable spectrophotometer, using dilute sulfuric acid (1 in 6) as the blank. Calculate the quantity in mg, of $C_{17}H_{20}N_2O_2$ in each mL of the Ophthalmic Solution taken by the formula $(C/V)(A_U/A_S)$, in which C is the concentration, in μg per mL, of USP Tropicamide RS in the Standard solution, V is the volume, in mL, of Ophthalmic Solution taken, and A_U and A_S are the absorbances of the solution from the Ophthalmic Solution and the Standard solution, respectively.

Crystallized Trypsin

» Crystallized Trypsin is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus* Linné (Fam. Bovidae). When assayed as directed herein, it contains not less than 2500 USP Trypsin Units in each mg, calculated on the dried basis, and not less than 90.0 percent and not more than 110.0 percent of the labeled potency.

NOTE—Determine the suitability of the substrates and check the adjustment of the spectrophotometer by performing the *Assay* using USP Crystallized Trypsin Reference Standard.

Packaging and storage—Preserve in tight containers, and avoid exposure to excessive heat.

Reference standard—*USP Crystallized Trypsin Reference Standard*—Keep container tightly closed, and store in a refrigerator. Allow container to reach room temperature before opening, and do not dry before using.

Solubility test—An amount, equivalent to 500,000 USP Trypsin Units, is soluble in 10 mL of water and in 10 mL of saline TS.

Microbial limit—It meets the requirements of the test for absence of *Pseudomonas aeruginosa* and *Salmonella* species and *Staphylococcus aureus* under *Microbial Limit Tests* ⟨61⟩.

Loss on drying ⟨731⟩—Dry it in vacuum at 60° for 4 hours: it loses not more than 5.0% of its weight.

Residue on ignition ⟨281⟩: not more than 2.5%.

Chymotrypsin—

0.067 M Phosphate buffer, pH 7.0—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 38.9 mL of the monobasic potassium phosphate solution with 61.1 mL of dibasic sodium phosphate solution. Adjust dropwise, if necessary, with dibasic sodium phosphate solution to a pH of 7.0.

Substrate solution—Dissolve 23.7 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in determining chymotrypsin, in about 50 mL of *0.067 M Phosphate buffer, pH 7.0* with warming. When cool, dilute with additional pH 7.0 buffer to 100 mL. (*Substrate solution* may be stored in the frozen state and used after thawing; it is important, however, to freeze immediately after preparation.)

Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 *N* hydrochloric acid to obtain a solution containing 650 USP Trypsin Units per mL.

Procedure—Conduct the test in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 0.1° in the cell compartment. Determine the temperature in the reaction cell before and after the measurement of absorbance to ensure that the temperature does not change by more than 0.5°. Pipet 200 μL of 0.0010 *N* hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance reads 0.200 at 237 nm. Pipet 200 μL of *Crystallized Trypsin solution* into another 1-cm cell, add 3.0 mL of the *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—This order of addition is to be followed.] At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for not less than 5 minutes. Repeat the procedure on the same dilution at least once. Absolute absorbance values are of less importance than the constancy of the rate of change of absorbance. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. The duplicate run at the same dilution should match the first run in rate of absorbance change. Determine the average absorbance change per minute, using only the values within the 3-minute portion of the curve where the rate of absorbance is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075 per minute under the conditions specified in this test. Calculate the number of USP Chymotrypsin Units per mg of Crystallized Trypsin by the formula $(A_2 - A_1)/(0.0075TW)$, in which A_2 is the absorbance straight-line initial reading, A_1 is the absorbance straight-line final reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbance. Not more than 50 USP Chymotrypsin Units per 2500 USP Trypsin Units is found, indicating the presence of not more than approximately 5% of chymotrypsin.

Assay—

0.067 M Phosphate buffer, pH 7.6—Dissolve 4.54 g of monobasic potassium phosphate in water to make 500 mL of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in water to make 500 mL of solution. Mix 13 mL of the monobasic potassium phosphate solution with 87 mL of the anhydrous dibasic sodium phosphate solution.

Substrate solution—Dissolve 85.7 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying Crystallized Trypsin (see NOTE), in water to make 100 mL. Dilute 10 mL of this solution with *0.067 M Phosphate buffer, pH 7.6* to 100 mL. Determine the absorbance of this solution, in a 1-cm cell, at 253 nm, in a suitable spectrophotometer equipped with thermospacers to maintain a temperature of 25 ± 0.1°, using water as the blank. By the addition of *0.067 M Phosphate buffer, pH 7.6*, or of the *Substrate solution* before dilution, adjust the absorbance so that it

measures not less than 0.575 and not more than 0.585. Use this *Substrate solution* within 2 hours.

Crystallized Trypsin solution—Dissolve a sufficient quantity of Crystallized Trypsin, accurately weighed, in 0.0010 *N* hydrochloric acid to obtain a solution containing about 50 to 60 USP Trypsin Units per mL.

Procedure—Pipet 200 µL of 0.0010 *N* hydrochloric acid and 3.0 mL of the *Substrate solution* into a 1-cm cell. Place this cell in a spectrophotometer, and adjust the instrument so that the absorbance reads 0.050 at 253 nm. Pipet 200 µL of *Crystallized Trypsin solution*, containing 10 to 12 USP Trypsin Units, into another 1-cm cell, add 3.0 mL of *Substrate solution*, and place the cell in the spectrophotometer. At the time the *Substrate solution* is added, start a stopwatch, and read the absorbance at 30-second intervals for 5 minutes. Repeat the procedure on the same dilution at least once. Plot a curve of absorbance against time, and use only those values that form a straight line to determine the activity of the Crystallized Trypsin. If the rate of change does not remain constant for at least 3 minutes, repeat the run, and if necessary, use a lower concentration. One USP Trypsin Unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified in this *Assay*. Calculate the number of USP Trypsin Units per mg by the formula $(A_1 - A_2)/(0.003TW)$, in which A_1 is the absorbance straight-line final reading, A_2 is the absorbance straight-line initial reading, T is the elapsed time, in minutes, between the initial and final readings, and W is the weight, in mg, of Crystallized Trypsin in the volume of solution used in determining the absorbances.

Crystallized Trypsin for Inhalation Aerosol

» Crystallized Trypsin for Inhalation Aerosol is prepared by cryodesiccation. It contains not less than 90.0 percent and not more than 110.0 percent of the labeled potency of trypsin.

Packaging and storage—Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.

Reference standard—*USP Crystallized Trypsin Reference Standard*—Keep container tightly closed, and store in a refrigerator. Allow container to reach room temperature before opening, and do not dry before using.

Identification—Prepare a substrate solution as follows: Transfer 7.7 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying trypsin crystallized, to a 100-mL volumetric flask. Add 20 mL of *0.067 M Phosphate buffer, pH 7.6*, prepared as directed in the *Assay* under *Crystallized Trypsin*, add 1 mL of methyl red–methylene blue TS, and dilute with water to volume. Mix 0.01 mL of this solution with 0.01 mL of a solution of Crystallized Trypsin for Inhalation Aerosol containing 250,000 USP Units in 6 mL of saline on a spot plate: a purple color is produced (*distinction from chymotrypsin, which produces no purple color within 3 minutes*).

Solubility test—Crystallized Trypsin for Inhalation Aerosol containing 500,000 USP Trypsin Units is soluble in 10 mL of water and in 10 mL of saline TS.

Assay—

0.067 M Phosphate buffer, pH 7.6 and *Substrate solution*—Prepare as directed in the *Assay* under *Crystallized Trypsin*.

Crystallized Trypsin solution—Dissolve the contents of one vial of Crystallized Trypsin for Inhalation Aerosol in 10.0 mL of 0.0010 *N* hydrochloric acid. Dilute this solution quantitatively with the same dilute acid to obtain a solution containing 50 to 60 USP Trypsin Units per mL.

Procedure—Proceed with Crystallized Trypsin for Inhalation Aerosol as directed for *Procedure* in the *Assay* under *Crystallized Trypsin*.

Tryptophan

$C_{11}H_{12}N_2O_2$ 204.23

L-Tryptophan.

L-Tryptophan [*73-22-3*].

» Tryptophan contains not less than 98.5 percent and not more than 101.5 percent of $C_{11}H_{12}N_2O_2$, as L-tryptophan, calculated on the dried basis.

Packaging and storage—Preserve in well-closed containers.

Reference standard—*USP L-Tryptophan Reference Standard*—Dry at 105° for 3 hours before using.

Identification—The infrared absorption spectrum of a potassium bromide dispersion of it, previously dried, exhibits maxima only at the same wavelengths as that of a similar preparation of USP L-Tryptophan RS.

Specific rotation ⟨781⟩: between −29.4° and −32.8°, calculated on the dried basis, determined in a solution containing 100 mg in each 10 mL. (Heat gently to dissolve, if necessary.)

pH ⟨791⟩: between 5.5 and 7.0, in a solution (1 in 100).

Loss on drying ⟨731⟩—Dry it at 105° for 3 hours: it loses not more than 0.3% of its weight.

Residue on ignition ⟨281⟩: not more than 0.1%.

Chloride ⟨221⟩—A 0.73-g portion shows no more chloride than corresponds to 0.50 mL of 0.020 *N* hydrochloric acid (0.05%).

Sulfate ⟨221⟩—A 0.33-g portion shows no more sulfate than corresponds to 0.10 mL of 0.020 *N* sulfuric acid (0.03%).

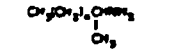
Arsenic ⟨211⟩: 1.5 ppm.

Iron ⟨241⟩: 0.003%.

Heavy metals, *Method II* ⟨231⟩: 0.0015%.

Assay—Transfer about 200 mg of Tryptophan, accurately weighed, to a 125-mL flask, dissolve in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with 0.1 *N* perchloric acid VS, determining the end-point potentiometrically. Perform a blank determination, and make any necessary correction. Each mL of 0.1 *N* perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

Tuaminoheptane



$C_7H_{17}N$ 115.22

2-Heptanamine.

1-Methylhexylamine [*123-82-0*].

» Tuaminoheptane contains not less than 99.0 percent and not more than 100.5 percent of $C_7H_{17}N$.

Packaging and storage—Preserve in tight containers, and store in a cool place.

Reference standard—*USP Tuaminoheptane Sulfate Reference Standard*—Dry at 105° to constant weight before using.

Identification—

Standard preparation—Dissolve 150 mg of USP Tuaminoheptane Sulfate RS in 5 mL of water. Render the solution alkaline to litmus with 1 *N* sodium hydroxide, and extract the solution with 2 mL of chloroform. Filter the chloroform extract through a layer of 2 g of granular anhydrous sodium sulfate supported on glass wool.

Procedure—The infrared absorption spectrum, determined in a 0.1-mm cell, of a 1 in 20 solution of Tuaminoheptane in chloroform exhibits maxima only at the same wavelengths as that of the *Standard preparation*.

Specific gravity ⟨841⟩: between 0.760 and 0.763.

Refractive index ⟨831⟩: between 1.415 and 1.417.

Nonvolatile residue—Weigh accurately about 1 g in a tared,

Annex 5.29

PHARMACEUTICAL ENZYMES

PROPERTIES AND ASSAY METHODS

EDITORS

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5. Assay Methods for Pancreatin

Pancreatin is a preparation containing enzymes having a protease, lipase and amylase activity.
It may be prepared from the fresh or frozen pancreas of certain domestic animals, mainly hogs.
The dried product may be diluted with lactose, sodium chloride or pancreatin of lower digestive power.
Pancreatin contains in 1 mg not less than 0.5 unit of free protease activity, not less than 1.0 unit of total protease activity, not less than 15 units of lipase activity and not less than 12 units of amylase activity.
Description
A white or buff coloured amorphous powder; free from unpleasant odour or taste.

5.1. IDENTIFICATION

Reagents
A. N Sodium hydroxide (Eur. Ph.)
B. Cresol red solution (Eur. Ph.)
C. Congo red (Eur. Ph.)
D. Congo red fibrin
Soak washed and shredded fibrin overnight in a 2.0 percent w/v solution of Congo red in alcohol (90 percent); strain, wash the fibrin with water, and store under solvent ether.
E. Solvent ether (Eur. Ph.) dried
F. Iodine 0.001 N
Dilute iodine 0.01 N (Eur. Ph.) ten times with water
G. Soluble starch (Eur. Ph.)

PANCREATIN

I. The proteolytic activity is rapidly destroyed in acid medium, by a 1% trituration in 0.05 N hydrochloric acid, (distinction from pepsin) and also all enzymic activity is destroyed by boiling water.
II. Triturate 1 g with 100 ml of water, adjust to pH 8.0 by the addition of N sodium hydroxide, using cresol red solution as indicator, and divide the liquid into two portions; boil one portion to destroy the enzyme. To each portion add a few shreds of Congo red fibrin, warm to 38°C to 40°C, and maintain at this temperature for one hour; the boiled liquid is only weakly stained red and distinctly less red than the unboiled one.
III. Triturate 1 g with 100 ml of water, adjust to pH 8.0 by the addition of N sodium hydroxide, using cresol red solution as indicator, and divide the liquid into two portions ; boil one portion to destroy the enzyme.
Add 2 g of soluble starch to 100 ml of boiling water, boil for two minutes, cool, and dilute to 150 ml with water.
To half of the cooled starch mucilage add the unboiled pancreatin liquid, and to the remainder the boiled pancreatin liquid, and maintain the mixtures at 39°C to 40°C for five minutes.
Transfer 1 ml of each mixture by means of a pipette to 10 ml of 0.001 N iodine; the unboiled liquid remains colourless and the boiled liquid acquires an intense blue colour.

Fat Assay

Place 2.0 g of pancreatin into a flask of about 50 ml capacity, add 20 ml of ether, close with stopper, and set it aside for 2 hours, mixing by rotating at frequent intervals.
Decant the supernatant ether by means of a guiding rod into a plain filter of about 7 cm diameter, previously moistened with ether, and collect the filtrate in a tared beaker.
Repeat the extraction with a 10-ml portion of ether, proceding as directed before, then with another 10-ml portion of ether and the remainder of the pancreatin to the filter.
Allow to drain, evaporate the ether spontaneously, and dry the residue at 105°C for 2 hours : the residue of fat obtained weights not more than 100 mg (5.0 percent).
Loss on drying: dry pancreatin in vacuo at 60°C for 4 hours : it looses not more than 5 percent of its weight.
Microbial contamination: A sample of 10 g of pancreatin may not contain any germ of Salmonellae, nor a sample of 1 g any germ of Escherichia Coli (see p. 243).

5.2. ASSAY OF ENZYME ACTIVITY

The activities of proteases, lipase and amylase represent essentially the digestive function of pancreatin (see procedures).

PHARMACEUTICAL ENZYMES

Units and standards

Unit of protease activity

The unit of protease activity is contained in that amount of the standard preparation which, under the conditions of the assay, hydrolyzes at an initial rate such that there is liberated per minute an amount of peptides not precipitated by trichloroacetic acid which gives the same absorbance at 275 nm, as one micromole of tyrosine.

Unit of lipase activity

The unit of lipase activity is contained in that amount of the standard preparation which under the conditions of the assay liberates one micro equivalent of fatty acid per minute.

Unit of amylase activity

The unit of amylase activity is that amount of pancreatin which under the conditions of the assay decomposes starch at an initial rate such that one micro equivalent of glucosidic linkage is hydrolyzed per minute.

Standards

Standards of protease and lipase are available at the Centre for Standards of the International Commission on Pharmaceutical Enzymes (Wolterslaan 12, Gent, Belgium).

5.2.1. Assay of protease activity

5.2.1.1. REAGENTS

A. Sodium hydroxide (Eur. Ph.) : 0.1 N

B. Hydrochloric acid (Eur. Ph.) : 0.1 N

C. Calcium chloride $2H_2O$ (Eur. Ph.) : 0.02 M
dissolve 2.94 g of calcium chloride $2H_2O$ in 900 ml water, adjust the pH to 6.0 - 6.2 and add sufficient water to produce 1000 ml, store in a cool place.

D. Borate buffer : dissolve 2.5 g of sodium chloride (Eur. Ph.), 2.85 g of disodium tetraborate $10H_2O$ (Eur. Ph.) and 10.5 g of boric acid (Eur. Ph.) in sufficient water to produce 1000 ml ; the pH must be 7.4-7.6. Store in a cool place.

E. Trichloroacetic acid (Eur. Ph.) : dissolve 50 g of the TCA acid in sufficient water to produce 1000 ml solution.

F. Enterokinase Organon (FIP controlled, assay on p. 77) : make a solution containing about 1 mg of enterokinase (containing at least 0.24 of enterokinase FIP units in one mg) per ml of calcium chloride solution C.

G. Casein substrate: use casein Merck A.G. nr. 2244 (FIP controlled). Suspend 1.25 g of casein calculated on an anhydrous base in 5 ml of water, add 10 ml of sodium hydroxide 0.1 N solution and stir for one minute, add 60 ml of water and stir with a magnetic stirrer until solution is clear, and adjust the pH to 8.0 with either sodium hydroxide or hydrochloric acid 0.1 N. The water content is determined by heating at 60°C in vacuo for 4 hours. Complete the volume by adding water to 100 ml. Use on the day of preparation.

H.Standard solution: triturate 100 mg of the pancreatin standard preparation in a cooled mortar with a portion of the cold calcium chloride solution C and transfer the trituration quantitatively in a 100 ml measuring flask by rinsing with several portions of a cold calcium chloride solution.

I. Diluted standard solution: dilute a portion of the standard solution H with cold borate buffer D in such a way that a final concentration of about 0.065 U/ml of activity is obtained (solution "S").

J. Sample solution: prepare in the same way as prescribed under H.

K. Diluted sample solution: mix 10 ml of the obtained solution J with 10 ml enterokinase solution F, warm and maintain the mixture at 35°C in a waterbath for 90 minutes.
Cool and dilute with cold borate buffer solution D to a concentration of about 0.065 U/ml and label this solution as "U".
Determine as prescribed under assay of total protease activity.

5.2.1.2. PROCEDURE FOR PROTEASE ACTIVITY

Label test-tubes in duplicate, S1, S2, and S3 for the standard series.
Test-tubes for the corresponding controls are labelled S1b, S2b and S3b also in duplicate.
Two tubes are labelled U and the corresponding controls Ub for the unknown sample.
Pipette into tubes S1 2.00 ml, into S2 and U 1.00 ml of buffer solution D.
Then pipette into the S1 1.00 ml, into S2 2.00 ml and into S3 3.00 ml of standard test dilution I.
Pipette into tubes U 2.00 ml of the diluted solution of the unknown K.
The corresponding controls S1b, S2b, S3b and Ub are treated in the same way.
Pipette into these controls 5.0 ml of trichloroacetic acid solution E and mix.
All the tubes, with a glass stirring rod in each, are put in a waterbath of 35°C for temperature equilibration.

Add to the controls 2.0 ml of casein substrate G and mix by stirring.
At zero time add to the tubes S1, S2, S3 and U at timed intervals 2.00 ml of the casein substrate G, preheated to waterbath temperature and mix immediately.
Exactly 30 minutes after the addition of the casein substrate, stop the reaction in tubes S1, S2, S3 and U by adding 5.00 ml of trichloroacetic acid solution E and mix thoroughly.
The tubes are removed from the bath and allowed to stand for about 20 minutes at room temperature for complete protein precipitation and are filtered twice through the same filter. The filtrate must be free from haze.
Determine the absorbance of the filtrate in 1 cm cells at 275 nm with a suitable spectrophotometer, using the filtrate of the reagent control (tube B) to set the instrument.
The reagent control consists of 3.00 ml of buffer solution D, 5.00 ml of trichloroacetic acid E and is further treated in the same way as the other controls.

Calculation of potency

Correct the mean absorbance value for the filtrates from tubes S1, S2 and S3 by subtracting the mean absorbance of the filtrates S1b, S2b and S3b respectively and plot these corrected values against the corresponding volumes of the standard test dilution used.
The corrected absorbance values should lie between 0.150 and 0.600.
Then a rectilinear dose-effect curve can be expected.
A tyrosine solution containing 0.1 micromole tyrosine per ml gives an absorbance of 0.116 at 275 nm. The potency of the sample solution is calculated by comparing its corrected absorbance value (U-Ub) with the plotted absorbance values for the standard test dilution. The activity of an unknown sample of pancreatin in units can be obtained by multiplying the ratio with the activity of the standard mentioned on the label. The activity is calculated using the formula :

$$\text{Act.} = \frac{1}{3.48} \times \frac{a}{b} \text{ FIP units per mg}$$

where a = absorbance of the filtrate at 275 nm
b = amount of pancreatin in mg.
This formula is derived from :

$$\text{Act.} = \frac{a}{0.116} \times \frac{10 \text{ (total test volume)}}{10 \text{ (ml equivalent to 1 micromole tyrosine)}} \times \frac{1}{30 \text{ (reaction time)}} \times \frac{1}{b}$$

where 0.116 = absorbance of 0.1 micromole/ml solution of tyrosine.

Example

When using casein Merck (Darmstadt) as a substrate, 0.090 mg pancreatin Reference Standard gives, when corrected for the control, an average absorbance of 0.505 from 10 ml filtrate. The potency is calculated as :

$$\text{Act.} = \frac{1}{3.48} \times \frac{0.505}{0.090} = 1.62 \text{ FIP units per mg}$$

and the unit of activity is 0.62 mg.

5.2.1.3. NOTES

1. free protease

The free protease content can be determined by direct diluting the sample solution J in the same way as prescribed under I.

2. suitability filterpaper

Determine the suitability of the filterpaper by filtering a 5 ml portion of trichloroacetic acid solution through the paper.
Measure the absorbance of the filtrate using an unfiltered portion as blank.
The absorbance is less than 0.04 for a cell of 2 cm path-length.

3. Activity of enterokinase

The activity can be determined as follows :

Principle

Pure trypsinogen is incubated with enterokinase under standard conditions at pH 6.0 - 6.2 and 35°C. The trypsin activity generated is measured by using the accepted method for trypsin.

Unit definition

One FIP unit of enterokinase activity is contained in that amount of the standard preparation, which under the specified standard conditions, forms one FIP unit of trypsin per minute.

Reagents and apparatus

A. $CaCl_2$ solution 0.02 M : dissolve 2.94 g of calcium chloride $2H_2O$ in 900 ml water, adjust the pH to 6.0 - 6.2 and add sufficient water to produce 1000 ml ; store in a cool place.

B. Substrate solution : pure trypsinogen Worthington (Type TG) is used. 16 mg accurately weighed is dissolved in 10.00 ml $CaCl_2$ solution A. The solution, formed after some time, does not become sparkling clear but stays hazy.

C. Enterokinase solution : a solution, containing 1 to 2 U/ml of enterokinase is prepared by dissolving the enzyme in the necessary amount of $CaCl_2$ solution A.

D. Borate buffer pH 8.0 : dissolve 5.72 mg borax ($Na_2B_4O_7 . 10 H_2O$) and 2.94 g of calcium chloride ($CaCl_2 . 2H_2O$) in approximately 800 ml of distilled water and titrate with N hydrochloric acid (approximately 2.5 ml) to pH 8.0. Dilute to 1 litre with redistilled water.

E. N-Benzoyl-L-arginine ethyl ester 0.02 M : dissolve 685 mg of N-benzoyl-L-arginine ethyl ester hydrochloride in 100 ml of distilled water.

F. Trypsin solution : prepare a solution containing approximately 50 FIP units per ml of trypsin by dissolving the enzyme in 0.001 N hydrochloric acid.

Waterbath

Apparatus for trypsin activity measurements : see monograph for trypsin.

Procedure

To the incubation vessel is added 4.5 ml $CaCl_2$ solution A, 0.5 ml enterokinase solution C and, starting the stop-watch, 1.0 ml trypsinogen solution B. The contents are mixed and the vessel is closed and placed in the waterbath at 35°C. After 5-10 minutes of incubation, a sample of 0.5 ml is pipetted off and added to the reaction vessel with BAEE solution E. The trypsin activity is measured according to the given procedure (p. 36). More samples are taken, after time intervals of about 7 and 14 minutes.

From these results one can calculate the trypsin units formed per minute by 0.5 ml of enterokinase solution.

When this solution contains between 1 and 2 U/ml reproducible results are obtained.

Calculation

These results are plotted graphically (trypsin units formed versus time of incubation) and the points lay (almost) on a straight line. From the slope of this line the trypsin units formed per minute in a 0.5 ml sample are calculated.

5.2.2. *Assay of lipase activity*

5.2.2.1. REAGENTS

Use only bidistilled or demineralized water.

A. Gum Arabic (Acacia Eur. Ph.) : 10 % solution

Dissolve 200 grams of gum arabic in 2000 ml of water.

Stir with a mechanical stirrer for 2 hours and then centrifugate at approx.

5000 r.p.m. for 30 minutes until clear or almost clear.
Store (freeze) this solution in containers of approx. 250 ml at −20°C.

B. Sodium taurocholate (FIP controlled) : 8 % (w/v) solution
Dissolve 2 grams of sodium taurocholate in water and dilute to 25 ml.
Prepare freshly every day.

C. Buffer solution
Dissolve 60.6 mg tris(hydroxymethyl)aminomethane p.a. (Merck Darmstadt, nr 8382 or equivalent quality) and 234.0 mg sodium chloride (Eur. Ph.) in water and dilute to 100 ml.
To be freshly prepared for 3 days (store in refrigerator).

D. Sodium hydroxide (Eur. Ph. or equivalent quality) : ± 0.10 N
Prepare a 50 % (w/v) solution of sodium hydroxide in water, allow to stand for a night and dilute the clear supernatant solution to 0.10 N with carbon dioxide-free water (prepared by boiling) and standardize to oxalic acid in accordance with standard specifications.

E. Olive oil, preferably produced by cold pressing and of B.P. 1973 quality.
Store in refrigerator.
Stock emulsion : transfer to a 800 ml (9 cm diameter) beaker 40 ml olive oil, 330 ml gum solution A and 30 ml water.
The rotor house is placed almost at the bottom of the beaker with the splash guard 15 mm above the rotor house and about 12 mm below the surface of the mixture.
Set the beaker in a pan containing ethanol with sufficient dry-ice as refrigerant. Switch on and stir with a medium speed of 1000 - 2000 r.p.m.
Cool down to -5 - 10°C. Increase the speed to maximum.
Mix for 30 min.
The temperature is kept below about 25°C by adding sufficient dry-ice to the refrigerant. A mixture of $CaCl_2$ and crushed ice is also suitable.
When stored in a refrigerator the stock emulsion can be used during 14 days.
A good emulsion may not show visible layers of separated oil.
90 % of the size of the droplets during this storage falls below 3 micron and none droplets have a diameter higher than 10 μm (controlled by microscope).
Shake vigorously before preparing a substrate emulsion.

F. Substrate emulsion (to be freshly prepared daily).
Prepare a quantity of substrate e.g. for 10 determinations by mixing the following substrates in the order indicated : 100 ml stock emulsion E, 80 ml buffer solution C, 20 ml sodium taurocholate solution B and 95 ml water.

Swirl the mixture after each addition or stir gently.

G. Lipase solvent
Dissolve 10 g sodium chloride, 6.06 g tris(hydroxymethyl)aminomethane and 4.90 g maleic anhydride (Merck Darmstadt, nr 408 is suitable) in 900 ml of water and titrate potentiometrically with 4 N sodium hydroxide to pH 7.0 (about 13 ml).
Dilute to 1000 ml with water. To be freshly prepared for 3 days (store in refrigerator).

H. Standard lipase: valid FIP standard (FIP controlled).
Store in deep-freezer (about —20°C).
Standard lipase suspension: take the bottle with standard from the freezer.
Allow the bottle to reach room temperature to prevent moistening by condensing water.
Open the bottle and weigh accurately an amount of powder containing about 5000 units of the lipase standard.
Triturate this intensively in a small cold mortar with the aid of 1 ml ice-cold lipase solvent G, dilute and transfer quantitatively by several rinsings with ice-cold lipase solvent G to a 200 ml volumetric flask.
Triturate in such a way that a very fine suspension is obtained.
Put the flask with enzyme solution in water with crushed ice.
Complete the volume with ice-cold lipase solvent G and mix.
After diluting to volume, carry out the determination immediately.
Allow the measuring flask with the enzyme solution to stand on ice during the determination.
For the determination use 0.5 ml containing about 12 units of lipase.

I. Pancreas powder suspension: accurately weigh off a quantity of pancreas powder, corresponding to approx. 5000 units, in a small mortar. Prepare a suspension as described for the preparation of the standard lipase suspension.

J. Ethanol, technical quality.

K. Dry ice or a mixture of calcium chloride and crushed ice.

L. Standard buffer of about pH 7.

5.2.2.2. APPARATUS

Titration equipment and pH-meter.
Thermostatted waterbath equipped with circulation pump, capacity about 3.5 litres per minute with a temperature of 37°C ± 0.1 C.
Chronometer divided in 60 seconds, subdivision 0.2 sec. or equivalent.

Mixer: only high speed mixers of about 0.25 HP and 8000 r.p.m. are suitable.
A fast mechanical or magnetic stirrer.
A manual method is prescribed; however to reduce the standard deviation a pH-stat method with a mechanically driven piston burette may be recommended.
Some measuring cylinders 50 ml high model.
1 Suction flask.
1 Conical flask to store ice-cold lipase solvent G.
1 Graduated quick delivery pipette.
1 Pan with a content of about 4 litres.
Some 200 ml volumetric flasks.
Some accurate thermometers.
Mortar, internal diameter about 50 mm.
High power microscope with an eye piece equipped with a calibrated micrometer.

5.2.2.3. KEYPOINTS

1. While preparing the stock emulsion the temperature may never rise over about 25°C.
2. $\geqslant$ 90% of the droplets of the oil emulsion have a diameter less than 3 μm and none greater than 10 μm.
3. Triturate the enzyme suspension carefully without lumps.
4. Place the pipette delivery tip in the substrate during the pipetting of the enzyme solution.

5.2.2.4. PROCEDURE FOR LIPASE ACTIVITY

A. **Standard lipase** (in triplicate).

Warm the waterbath beforehand in such a way that the temperature of 29.5 ml substrate in the incubation vessel becomes 37°C $\pm$ 0.1°C.
Empty after each test the incubation vessel by suction with the aid of the suction flask and vacuum and rinse with a few portions of water, removing the rinsings by suction each time.
Standardize the pH-meter with the aid of a N.B.S. standard buffer or equivalent (in the range of pH 7).
Measure 29.5 ml substrate F in a 50 ml measuring cylinder.
Place the measuring cylinder in the waterbath for at least 10 min. to pre-warm it and then pour the substrate into the incubation vessel.
Place the electrodes, stirrer and hydroxide delivery tube in the vessel so that the mounted lid covers the vessel. Switch on the apparatus.
Start the stirrer and carefully add hydroxide solution D until the pH is approx. 9.2.

Fill a micrometer syringe with 0.1 N sodium hydroxide and set it to 0.
With a calibrated quick-delivery graduated pipette accurately pipette approx. 0.5 ml of previously homogenized standard suspension H into the incubation vessel and start at the same moment the chronometer and add continuously from the micrometer syringe sufficient 0.1 N sodium hydroxide to maintain the pH at 9.0.
Record after exactly 1 minute the amount of 0.1 N sodium hydroxide consumption.
Repeat these readings another 4 times.
Neglect the first reading and determine the mean of the other 4.
Repeat the assay procedure twice.
Call the mean values S1, S2 and S3; the quantity of hydroxide required is on average approx. 0.12 ml 0.1 N NaOH/minute (range 0.08-0.16 ml).

B. **Sample of pancreas powder** (in duplicate).

Determine the lipase activity of the pancreas suspension I in the same way as for the standard lipase.
If the quantity of hydroxide required differs considerably from the quantity of hydroxide required for the standard (approx 0.12 ml/min., range 0.08 - 0.16 ml), the determination should be repeated with an adapted quantity of pancreas powder suspension.
This should be within the range of 0.4 - 0.6 ml, or the quantity of pancreas powder should be adjusted in order to be able to satisfy the assay conditions.
Call the mean values M1 and M2.

Calculation

$$\frac{M}{S} \times \frac{Gs}{Gm} \times \text{ref. St.} = \text{units/mg with reference to the FIP standard}$$

where: M = mean value of M1 and M2 in 0.1 N sodium hydroxide soln.
S = mean value of S1, S2 and S3 in 0.1 N sodium hydroxide solution.
Gs = weight of lipase standard weighed out in mg.
Gm = weight of pancreas sample weighed out in mg.
ref. St. = potency of a valid FIP standard in FIP units/mg.

The assay on the substance being examined is only valid if the determined potency of the standard preparation is not less than 70 percent and not more than 130 percent of its declared potency. Put standard lipase into the deep-freezer (about —20°C).

5.2.3. Assay of Amylase activity

5.2.3.1. REAGENTS

A. 0.2 M phosphate buffer solution pH 6.8
Mix 51 ml of 0.2 M potassium dihydrogen phosphate solution with sufficient 0.2 M disodium hydrogen phosphate to produce about 100 ml of a buffer solution with a pH of 6.8.
B. Amylum solubile Merck (Darmstadt nr 1252 FIP controlled)
Starch substrate solution :
Stir an amount of FIP controlled starch equivalent to 2.0 g of dried substance with 10 ml of water.
The water content is determined by heating at 120°C for 4 hours.
Add with continuous agitation to 160 ml of boiling water.
Rince the starch container with a further 10 ml of water, add the washings to the hot starch solutions and heat to boiling while stirring continuously.
Cool to room temperature and add sufficient water to produce 200 ml.
C. 0.1 N Sodium hydroxide (Eur. Ph.)
D. Sulfuric acid (Eur. Ph.)
E. 0.1 N Sodium thiosulfate (Eur. Ph.)
F. N Hydrochloric acid (Eur. Ph.)
G. 0.2 M Sodium chloride
H. 0.1 N Iodine solution

5.2.3.2. PROCEDURE FOR AMYLASE ACTIVITY

Triturate an amount of the substance being examined equivalent to approx. 1500 units of amylase activity with 60 ml of 0.2 M phosphate buffer solution A for fifteen minutes and add sufficient 0.2 M phosphate buffer solution A to produce 100 ml (the potency of the solution must contain 10-20 units per ml).
To a stoppered tube, 22 mm in diameter and 200 mm long, add 25.0 ml of starch substrate B, 10.0 ml of 0.2 M phosphate buffer solution A and 1.0 ml of 0.2 M sodium chloride solution G.
Stopper the tube, mix the contents, and place in a waterbath at 25°C. When the temperature of the mixture has reached 25°C, add 1.0 ml of the solution of the substance being examined and record the time of addition.
Mix thoroughly and replace in the waterbath. After exactly 10 minutes add 2 ml of N hydrochloric acid F to stop the reaction. Transfer the contents of the tube to a 300 ml stoppered flask, rinse the tube with 20 ml of water, and add the washings to the flask. While stirring continuously add 10.0 ml of 0.1 N iodine H and immediately 45.0 ml of 0.1 N sodium

hydroxide C.
Allow to stand in the dark at a temperature between 15°C and 25°C for fifteen minutes. Add 4 ml of a mixture of one volume of sulfuric acid D and four volumes of water and titrate with 0.1 N sodium thiosulfate E. Repeat the procedure but add the 2 ml of N hydrochloric acid F before the addition of the solution ot the sample being examined.

Calculation of potency :
The amylase potency of the sample being examined is calculated from the empiric expression :

$$\frac{100}{(\frac{1}{5\,(b-a)} - 0.006)\,W} = \text{units per mg}$$

where : a = ml of 0.1 N sodium thiosulfate used in the titration of the substance being examined,
b = ml of 0.1 N sodium thiosulfate used in the titration of the substance being examined inactivated by the addition of N hydrochloric acid,
W = total weight in mg of the substance being examined in the solution prepared for assay.

Purification

The procedure developed by Herriott (15) for the crystallization of porcine pepsinogen depends largely on fractional precipitation with $(NH_4)_2SO_4$ and yields a product that is homogeneous by several criteria (16-18). Crystalline pepsin preparations are obtained by fractional precipitation with $MgSO_4$ (15, 19) or by crystallization from alcohol (21); as indicated above, such products are heterogeneous with respect to their behaviour on ion exchange columns.
A significant advance, however, was made by Rajagopalan et al. (18), who have used hydroxyapatite for the fractionation of commercial preparations of crystalline pepsin. They have described a valuable method for the preparation of pepsin samples that are homogeneous by chromatography on hydroxyapatite and by end group analysis; this method involves rapid activation of pepsinogen, and passage of the activation mixture through sulfoethyl Sephadex C-25 to remove the activation peptides, followed by desalting with Sephadex G-25. This procedure has been modified by the use of a long column of sulfoethyl Sephadex C-25 (22).

2. Pharmacological properties and therapeutic applications

Pepsin for pharmaceutical purposes is usually a relatively crude preparation. None of the pharmacopoeiae in which pepsin is the subject of a monograph, specifies pepsin of the purity of crystalline pepsin. This is the reason why pharmaceutical pepsin is so badly defined and no properties can be specified other than the determination of activity. It seems highly desirable that pure crystalline pepsin should be described in the International Pharmacopoeia as a reference preparation.
Pepsin may be administered in acid solution to increase the digestive power of gastric juice, particularly where there is a deficiency of pepsin secretion. Crystalline pepsin is used as a research tool in protein analysis, and as a standard in pepsin assay work particularly for the determination of pepsin in gastric juice.

3. Assay

3.1. PRINCIPLE

The most widely used assay method for pepsin activity is that developed by Anson (23), using hemoglobin as substrate. Here the trichloroacetic acid filtrates of the digested hemoglobin are treated with the Folin-

Ciocalteu phenol reagent; the blue colour developed due to tyrosine and tryptophan is measured colorimetrically, or the tyrosine is estimated directly by the absorption in the ultraviolet light at 275 nm. The here proposed assay method uses hemoglobin, with a modified Anson procedure. Because of the variability of the crude pharmaceutical grade of pepsin preparations, it was advisable to relate the found activities of these preparations by reference to a crystalline pepsin standard preparation. As even highly purified hemoglobin substrate preparations of different origin may give divergent results, it is necessary to conduct the assays in parallel on the crystalline pure and crude pepsin samples with the same hemoglobin. Activities should be expressed in relationship to the activity found for a reference standard of crystalline pepsin internationally distributed by the Commission.

3.2. UNIT DEFINITION

One FIP unit of pepsin activity is contained in that amount of the standard preparation, which upon incubation at 25.0°C ± 0.1°C for one minute with a suitable preparation of pure hemoglobin will cause the decomposition of the hemoglobin to such an extent that the amount of hydroxyaryl substances liberated will, upon reaction with Folin-Ciocalteu reagent, result in the formation of a coloured solution of equal intensity to that, resulting from the reaction of 1 micromole of tyrosine with the reagent.

3.3. REAGENTS

A. *4 percent Trichloroacetic acid solution*
Weigh 40.0 g of trichloroacetic acid, transfer to a 1000-ml volumetric flask and fill to the mark with water. This must be standardized to ± 0.1 per cent using 0.1 N sodium hydroxide solution.

B. *3.85 N Sodium hydroxide solution*
Weigh 15.4 g of sodium hydroxide, transfer to a 100-ml volumetric flask and fill to the mark with water.

C. *Phenol reagent stock solution* (C. Folin and V. Ciocalteu, J. Biol. Chem., *73*, 629, 1927)
Transfer 100.0 g of sodium tungstate ($Na_2WO_4.2H_2O$) and 25.0 g of sodium molybdate ($Na_2MoO_4.2H_2O$) together with 700 ml of water to a 1500-ml Florence flask. Add 50 ml of a 85 per cent phosphoric acid solution and 100 ml of concentrated hydrochloric acid and connect the flask to a reflux condenser. If ground glass joints are not used, wrap the cork of rubber stop with tinfoil. Boil the solution gently for 10 hours. Add

150.0 g of lithium sulfate (Li_2SO_4), 50 ml of water, and about 1 ml of liquid bromine. Boil without the condenser (under the hood) for 15 minutes to remove excess bromine. Cool, transfer the solution to a 1000-ml volumetric flask, dilute to the mark with water, mix well, and filter. The finished reagent should have no greenish tint, since this indicates the presence of blue reduction products. The solution should be kept well protected against dust, as organic materials will gradually produce slight reduction.

D. *2 per cent Hemoglobin substrate solution*

Since the purity of hemoglobin powder varies with each batch, a quantity large enough to last for some time should be acquired. Determine the purity by finding the nitrogen content (use the semi-micro Kjeldahl method with the mercury catalyst). The nitrogen content of pure bovine hemoglobin is 17.7 per cent. For example, if the desired amount is 20.0 g, use the following equation to find the necessary weight:

$$\frac{17.7 \times 20.0}{\text{nitrogen content found}} = \text{required weight of powder in gram.}$$

Transfer the above amount to a 200-ml filter flask. Add 20 ml of 0.06 N hydrochloric acid, apply a vacuum and shake the flask until the hemoglobin is in complete solution. Remove the vacuum and add the remaining 980 ml of 0.06 N hydrochloric acid. Add 0.250 g of merthiolate as a preservative. This solution must be kept refrigerated.

E. *Hydrochloric acid solution 0.03 N*

Measure 60 ml of N hydrochloric acid, dilute with sufficient water to make 1 litre and adjust to pH 1.6 ± 0.1.

F. *Preparation of sample solutions*

On the basis of the estimated potency dilute an appropriate size sample to a concentration of approximately 0.5 unit per ml. Dissolve the sample in 0.06 N hydrochloric acid and determine the pH of the solution. The pH of the solution should be 1.6 ± 0.1. If necessary adjust the pH to 1.6 with N hydrochloric acid before diluting to volume. Dissolve and dilute the sample solutions with 0.06 N hydrochloric acid just before the assay. Foaming of pepsin solutions during the dissolution of the sample should be avoided as the enzyme may rapidly be inactivated by surface denaturation.

G. *Preparation of standard reference solution*

Less than 15 minutes before the assay prepare a solution of 0.5 units per ml of the standard reference preparation in 0.06 N hydrochloric acid solution. Avoid shaking.

H. *Standard tyrosine solution*

Weigh 60.4 mg of pure tyrosine, dissolve in 0.1 N HCl and make up to 1000 ml with 0.1 N HCl.
This solution contains 0.1812 mg (or 1 micromole) per 3 ml.
Pipette 3 ml aliquots into 20 ml distilled water and mix.
Add 1 ml of 3.85 N NaOH and 1 ml of Folin-Ciocalteu reagent. After 15 minutes read the absorbance at 540 nm against a blank of 23 ml distilled water, 1 ml 3.85 N NaOH and 1 ml Folin-Ciocalteu reagent.

3.4. PROCEDURE

Place flasks of 4% trichloroacetic acid (sol. A) and the substrate hemoglobin (sol. D) in the waterbath to equilibrate at 25°C. Arrange a rack of three test tubes for the standard solutions marked: S_1, S_2, S_3 and one blank B_s, provided with footed stirring rods and place this in the 25°C waterbath.
Arrange also a rack of three test tubes for the test solutions marked I_1, I_2, I_3 and one blank B_t in the same way. Transfer into test tubes S_1, S_2, S_3 and B_s 1.0 ml of the standard solution G and into test tubes I_1, I_2, I_3 and B_t 1.0 ml of the test solution F.
Equilibrate to 25°C.
To the test tubes B_s and B_t add 10.0 ml of the trichloroacetic acid solution A.
At zero time add 5.0 ml of substrate hemoglobin solution D successively and at time intervals of 30 seconds to test tubes S_1, S_2, S_3, I_1, I_2 and I_3.
Mix the solution by gently stirring.
Add also 5.0 ml of substrate hemoglobin solution D to test tubes B_s and B_t and mix.
Exactly 10 minutes after adding substrate and at the same time intervals stop the reaction by adding 10.0 ml trichloroacetic acid solution A to the test tubes S_1, S_2, S_3, I_1, I_2 and I_3 and mix.
Filter the samples and blanks through Whatman No. 2 filter paper and eliminate 5 ml of the first running filtrate.
Transfer 3 ml aliquots of the filtrate into tubes containing 20 ml water. Mix.
Add to each tube 1 ml of 3.85 N NaOH (solution B) and develop the colour by adding 1 ml of Folin-Ciocalteu reagent (solution C) to each tube, beginning with the blank and then the sample duplicates of each set, in a definite order.
After at least 15 minutes read the sample transmission of solutions S_1, S_2, S_3 and I_1, I_2, I_3 at 540 nm under a pathlength of 1 cm, using the respective blanks as 100 percent transmission references. The absorbance of the

solutions should be between 0.300 and 0.400. Calculate the mean value of absorbance of solutions S_1, S_2, S_3 (= E_s) and I_1, I_2, I_3 (E_t).

3.5. CALCULATION

If E_s = mean absorbance of the standard reference solution of pepsin,
E_t = mean absorbance of the test solution,
C_s = concentration of the standard reference solution of pepsin, in mg/ml,
C_t = concentration of the test solution, in mg/ml,
X_s = activity, in units, of the standard reference,
X_t = activity, in units per mg, of the sample,

$$\text{then } X_t = \frac{E_t \times C_s}{C_t \times E_s} \times X_s$$

The activity of the standard reference of pepsin is obtained by comparing the absorbance E_s to the solution of tyrosine standard.

If S = absorbance of the standard reference solution,
T = absorbance of the standard solution of tyrosine,
C = concentration of the enzyme solution, in mg/ml,
X = units per mg of the pepsin standard,

$$\text{then } X = \frac{16S}{30CT}$$

The absorbances of the tyrosine standard and test solutions should be in close agreement with one another and not differ more than ± 10 percent. If there is a wide divergence between the absorbances of the standard and the test solution then a lower value is obtained.

The absorbance of the standard solution must be determined for each running test because of the slight differences which may be observed, due to the alkaline and Folin reagent solution.

The activity required for pharmaceutical pepsin should fall between 0.5 and 0.7 units per mg.

Pepsin is described as a white or slightly yellowish, crystalline or amorphous, hygroscopic powder with a faint odour. It is soluble in water giving a clear or a slightly opalescent and weakly acid solution and insoluble in alcohol or ether. It should not contain more than 5 percent water and kept in a drying vessel.

A sample of 10 g pepsin may not contain any germ of Salmonellae, nor a sample of 1 g any germ of Escherichia Coli.

3.6. NOTES

a. *Identification test*

Introduce 1 ml of shreds of precipitated Congo red fibrin on a paper filter

and wash it out with a 0.06 N hydrochloric acid solution till a colourless filtrate is obtained. Perforate the filter paper and wash the Congo red fibrin through it with 20 ml of the same acid solution in a conical flask. Shake before use.

Dissolve a minimum content of pepsin of 20 units in 2 ml water and adjust to pH of 1.6 (± 0.1). Mix 1 ml of this solution in a test tube with 4 ml of the Congo red fibrin suspension and put in a waterbath at 25°C with gently shaking. Prepare a same test solution containing 1 ml water and put in the waterbath.

After 15 minutes of incubation the reference solution is colourless and the test tube with pepsin is blue violet.

b. Control work has been performed on the feasibility of the assay method. Difficulties may occur due to the standard pepsin even three times crystallized may contain impurities, known as pepsin B and C. The standard preparation showed a broad pH optimum of proteolysis running from pH 1.55 to 2.80 (De Nève, (8)).

pH	Absorb. 280 nm
1.55	0.910
2.06	1.000
2.64	0.980
2.80	0.950
3.24	0.700
3.62	0.356

In order to find out whether impurities of analogous enzyme molecules were interfering, denaturation was performed by heating to 48°C at pH 5.8 and by increasing pH to 7.1 at 25°C. The ratios of decrease of the activity assayed between pH 1.6 and 2.80 were running completely in parallel and there was in this way no indication of any foreign enzyme impurity acting here.

It is known that pepsin homologues B and C or parapepsins A and B produced by autodigestion) behave differently during heating and pH denaturation.

One source of error may be the time of incubation (10 min) with a variation from 10 to 12 seconds. Proteolysis times taken over 1, 4, 7, 10 minutes show a linear relationship of activity, with an intersection at the origin at 10-12 seconds. The reason was found to be the time required for stopping the enzyme reaction by the addition of trichloroacetic acid solution. Pipette emptying requires ca. 15 seconds, and proteolysis seems to be completely stopped when a concentration of 2% TCA is obtained. This is only obtained 10 seconds after addition of the solution. For this reason a fast running or blow out pipette is recommended.

Objections were raised that the wavelength of 540 nm used for the absorbance measurement was not the peak of the absorption curve that is situated at 740 nm. It was stated by R.B. Christie that in fact this absorption curve is very shallow so that the shift from the maximum of absorbance is fairly non substantial. Such a manoeuvre will of course reduce somewhat the sensitivity of the assay but in the case of the hemoglobin substrate technique, reproducibility for a pharmaceutical product is of greater importance.
Notice will be taken that results are also obtained and calculated by reference to a parallel running controlled standard preparation that further reduces possible errors due to measurement away from A maximum.

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Annex 5.30

Apparatus and Glassware Recommended for the Quality Control Unit of the Experimental Centre of Applied Enzymology and Microbiology

1. Ultraviolet-Visible Spectrophotometer, with thermostat, manual, SP-8620, Pye-Unicam, Cambridge, Great Britain.
2. pH-Meter, with a set of 10 spare glass electrodes, Radiometer, Copenhagen, Denmark.
3. Thin-Layer Chromatography Set, Camag, Muttenz, Switzerland.
4. Ultraviolet Lapm, for detection of spots in thin-layer chromatography, Camag, Mutterz, Switzerland.
5. Multidozimat, Type 655, for determination of lipase activity, Deputy Donau Trading AG, Binz Str. 7, CH-8045 Zürich, Switzerland.
6. Karl Fischer Apparatus, for water content determination, Radiometer, Copenhagen, Denmark.
7. Soxhlet Extraction Apparatus, for determination of fat content.
8. Kjeldahl Flasks (for determination of nitrogen content).

Annex 5.31.1

Manual and Pharmacopoeias Recommended for the Quality Control Unit of the Experimental Centre of Applied Enzymology and Microbiology

Manual

1. R. Ruyssen and A. Lauwers, Pharmaceutical Enzymes, E. Story-Scientia P.V.B.A., Scientific Publishing Co., Gent, Belgium, 1978.

Pharmacopoeias

2. International Pharmacopoeia, Third Edition, Two Volumes, World Health Organization, Geneve, 1981.

3. European Pharmacopoeia, Second Edition, Ten Volumes, Maisonneuve S. A., 57 Sainte-Ruffine, France, 1981-1986.

4. The United States Pharmacopoeia, Twenty-First Revision, Official from January 1, 1985, United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, Md. 20852, U.S.A.

Annex 5.31.2

RO GALENIKA
RJ ISTRAŽIVANJE I RAZVOJ,
INŽENJERING I KONTROLA KVALITETA
SEKTOR KONTROLE KVALITETA

BEOGRAD, 15. 06. 1987 god.

Kontrola br. 372032

Šifra

Uzorak ...IN IN BULK

Primljen od ________ pod brojem ________

Broj ulaza ________ broj zaključnice ________

Proizvođač (dobavljač) ... - Mongolia

Rok važnosti

Serija (šarža) 192 količina

533/311400 NALAZ br. 372032

Ispitivanja vršena prema: ... Internal specification

Određivanje	Rezultat
Description	Cream colored powder
Total proteolitic activity	2000 Armour units/mg
Trypsin activity	... US units/mg
Chymotrypsin activity	45. USP units/mg
Total bacterial count	10/g
Mould	05/g
Fungi	10/g
Pseudomonas aeruginosa:	Absent
Staphylococcus aureus	Absent
Enterobacteriaceae	Absent
Escherichia coli	Absent
Salmonella	Absent

Zaključak:

IND.

RADNA ORGANIZACIJA GALENIKA ... I RAZVOJ, INŽ... KONTROLA KVALITETA ... BEOGRAD — ZEMUN

Obr. 212

RUKOVODILAC SEKTORA

O. Lebanović

CERTIFICATE OF ANALYSIS

PANCYPSIN IN BULK
USP XXI and Internal specification

Batch No:	872832 (1c g)
Ser.:	192
Description:	Cream coloured powder
Total proteolitic activity:	26oo Armour units/mg
Trypsin activity:	23oo US Punits/mg
Chymotrypsin activity:	45o USP Units/mg
Total bacterial count:	1o/g
Mould:	35/g
Fungi:	1o/g
Pseudomonas aeruginosa:	Absent
Staphylococcus aureus:	Absent
Enterobacteriaceae:	Absent
Escherichia coli:	Absent
Salmonella:	Absent

Date: June 15, 1987.

QUALITY CONTROL DEPARTMENT
V. Laban-Božić
Dr Olivera Laban-Božić
Q.C.Manager

RADNA ORGANIZACIJA
GALENIKA
— ZEMUN

E C A E M	Raw material specification
Experimetal Centre of Applied Enzymology and Mycrobiology ULAN BATOR, MONGOLIA	Approved by:
	List nº
Pancypsin	Signature:

S P E C I F I C A T I O N

PANCYPSIN	Mixture of trypsin and chymotrypsin
QUALITY	USP and INTERNAL SPECIFICATION
DESCRIPTION	Cream Colored powder
ENZYMATIC ACTIVITY	
TOTAL PROTEOLITIC ACTIVITY	min. 2300 Armour units/mg
TRYPSIN	min. 2000 USP units/mg
CHYMOTRYPSIN	min. 400 USP
TRYPSIN/CHYMOTRYPSIN RATIO	5:1 - 7:1
SULPHATE	1%
LOSS ON DRYING	max 5%
RESIDUE ON IGNITION	max 3%

MICROBIAL CONTENT LIMIT

TOTAL BACTERIAL COUNT	max 5.10^4/g
MOULD and FUNGI	max 500/g
FREE OF PATOGEN BACTERIAL (E.Cola,Salmonell,Pseudomonas...)	Absent

P R E P A R E D:

V. Čobanović

(dipl.hem.spec, Milka Čobanović)

Annex 5.33

Explanation of the Reasons for the Products Chosen for the Project Second Phase

Only a few products are recommended for the production in the beginning of the Project Second Phase, mainly the products with technology and quality control elaborated in ECAEM.

- Pancypsin. This is the main product of ECAEM, well run on pilot plant scale and of excellent quality. Approximately 35 kg of Pancypsin was produced last year (1986). The new quality control methods were introduced during the Quality Control Expert's mission. ECAEM is producing Pancypsin for pharmaceutical usage in form of 25 mg sterile powder in vials and in bottles of 5 g and 100 g substance. The problem is in the small consumption in Mongolia. The experts advice was to increase the Pancypsin production by using the cattle pancreas besides the sheep and goat pancreas, and sell it abroad preferentially as substance in bulk. Pancypsin is an interesting product for the world market as antiinflammatory drug, but an economic survey is needed for that purpose. According to the Expert Technologist it is easier to sell Pancypsin than chymotrypsin and trypsin.

- Chymotrypsin, and

- Trypsin. In ECAEM they produce both enzymes from cattle and yak pancreas and they have the technology elaborated and introduced. The production is run on the same equipment as the Pancypsin production. The new quality control methods were introduced during the experts' mission, and both enzymes were of very high activity. They are being produced mainly as substance in bulk, but a certain amount of chymotrypsin and trypsin can be made also as sterile powder in vials. According to the Expert Technologist, trypsin in bulk can be easily sold on the world market, but with chymotrypsin sales some problems might occur. As both enzymes, as well as Pancypsin, are produced using the same equipment, it is quite easy to change the production plan to suit the existing situation.

- Dry bile. It was produced in the amount of 4.5 tons in 1986 and completely sold abroad. This is a well introduced product in ECAEM. However, a new quality control method is needed as soon as possible. An increase of quality and possible elaboration of technology for purification of dry bile to bile acids is recommended in the near future.

- Pancreatin. The laboratory scale experiments were done and a product of a very good quality was obtained. Full quality control was performed (for all three pancreatin enzymes), and the quality control methods introduced, by the Quality Control Expert. A good experience has to be gathered in the bigger scale production, as soon as possible. It is a real possibility of selling a certain amount of pancreatin for local consumption, but a bigger quantity will be desirable for sale abroad. An economic survey is needed in any case, as soon as possible.

- Medical grade pepsin. The medical pepsin is produced from pig stomach mucose. ECAEM has technology developed and tested on the laboratory scale, and they need experience in production on the pilot plant scale. The quality control was introduced during the experts mission, and pepsin of very good quality was found. The possibility of selling it on the world market are relatively good, according to the Expert Technologist's opinion.

- Food grade pepsin. The food grade pepsin is produced from cattle stomach mucose. ECAEM need more experience in this production, starting with the laboratory scale experiments. This pepsin is usually used for cheese processing. The pepsin activity expressed as the milk clotting test has to be introduced as well. During the mission, CTA found an interest in cheese production by pepsin in the Scientific-Experimental Centre of the Foodstuff Industry in Ulan Bator. For the beginning a relatively small amount of pepsin could be used for the local consumption, about 50 kg per year. A bigger amount might be sold abroad. In any case, an economic survey is needed for this.

- Peptone. In ECAEM they have a certain experience in the laboratory scale production of peptone, using paunch of cattle as raw material. They need experience in a larger scale production. As the quality of this peptone is mediocre, some problems may occur in selling it on the world market, as the Expert Technologist says. An economic survey is needed.