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TECHNICAL ADVISER FOR INSULIN EXTRACTION,

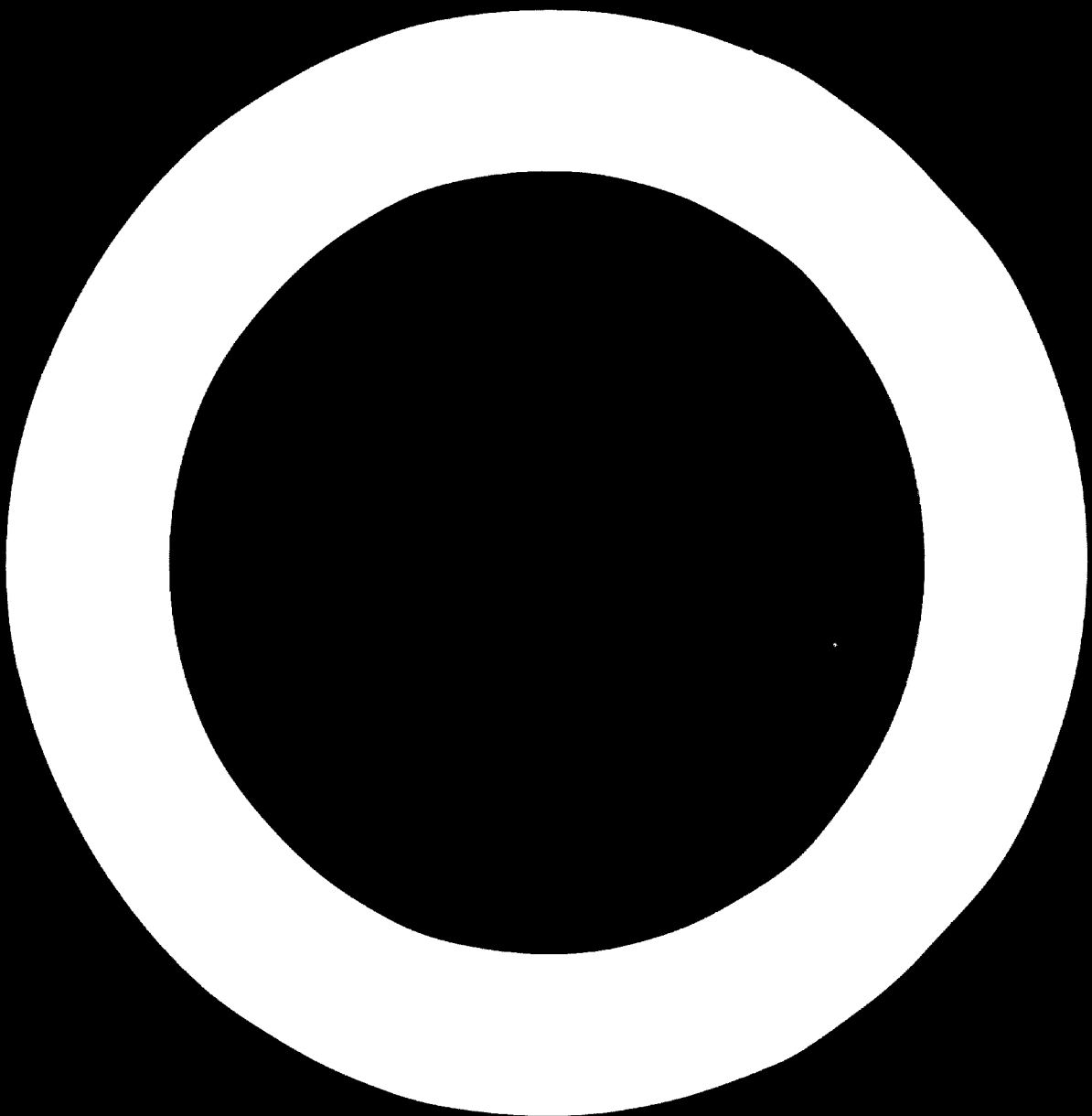
ES/BUR/74/002

BURMA .

Terminal Report .

Prepared for the Government of Burma by the
United Nations Industrial Development Organization,
executing agency for the
United Nations Development Programme

United Nations Industrial Development Organization



United Nations Development Programme

TECHNICAL ADVISER FOR
INSULIN EXTRACTION

(IS/BUR/74/002)

B U R M A

Project findings and recommendations

Prepared for the Government of Burma
by the United Nations Industrial Development Organization,
executing agency for the United Nations Development Programme

Based on the work of Oleg Scedrov, expert in insulin extraction

United Nations Industrial Development Organization
Vienna, 1975

Explanatory notes

The following symbols have been used throughout the report:

A comma (,) is used to distinguish thousands and millions.

A slash (/) indicates a field-season covering part of two consecutive years, e.g., 1974/75.

Use of a hyphen (-) between years, e.g., 1974-1976, signifies the full period involved, including the beginning and end years.

References to "dollars" indicate United States dollars, unless otherwise stated.

The following exchange rates are used in the conversion of country currencies to United States dollars:

Country	Currency	Exchange rate per US dollar in 1975
Burma	Kyat (K)	10.53

Acronyms

AMSD Army Medical Stores Department
BPI Burma Pharmaceutical Industry
CMSD Central Medical Stores Depot
CRO Central Research Organization
TCIO Trade Corporation IO

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INTRODUCTION

Insulin and its production

Insulin is a hormone which principally regulates the metabolism of carbohydrates and fats in vertebrates. Its deficiency causes diabetes mellitus. Insulin originates in the pancreas, secretes the hormone in the blood, and in severe cases is the only successful treatment. Administered by daily injections, it cannot cure diabetics, but it will maintain their well-being.

Insulin can be synthesized, but the process is very costly. On an industrial scale it is prepared exclusively from slaughter-house animals.

Project background

The Central Research Organization (CRO) at Rangoon has been granted permission by the Government of Burma for research during the period 1972-1978 into insulin production from domestic beef pancreas (CRO project number 2/[illegible], dated 1 April 1972). A total of K 212,000 will be spent on this project. The reasons for the undertaking are difficulties of transport and storage (insulin injections must be kept at temperatures between 2°C and 10°C) as well as the inadequate volume of imports. Due to CRO having had difficulties with insulin preparation, a request was made in early 1974 for a UNIDO technical adviser on insulin extraction to assist CRO in its laboratory-scale extraction and in increasing the capacity of production. CRO is partially equipped for insulin production and employs a chemist experienced in experimental extraction from beef pancreas.

Official arrangements

The project "Technical Adviser for Insulin Extraction" (IS/BUR/74/002) was authorized by the United Nations Development Programme (UNDP) in March 1974 and arrangement made for a contribution of $6,000. The job description of the expert is attached as annex I. The starting date of the project was 17 March 1975 and the completion date 16 May 1975; the stay of the expert in Rangoon lasted from 22 March to 10 May (annex II).

Project objectives

The objective of the project was to prepare the way for obtaining insulin injections from domestic sources. The import of insulin into Burma being less

than its requirements, the success of the project will partially eliminate this problem. The capacity of domestic production will probably not be high enough to have a positive financial effect. The significance of the project lies in the social gesture of helping those patients who otherwise could not survive or whose lives would be shortened for lack of regular insulin supply. Since in all human societies there exists a certain percentage of diabetics, every country needs insulin. For Burma it would be useful to become independent of importation of this product.

Basic requirements

The first and foremost necessity for the successful production of insulin injections from domestic sources is to have an established method for biological assay of the insulin produced. Then it is necessary to find an appropriate enterprise which will be able to produce insulin injections. Only after the solution of these problems is it possible to consider the production of crystalline insulin from the pancreas of slaughter-house animals on an industrial or semi-industrial scale. The first step is to develop a method for obtaining insulin on a laboratory scale.

To be able to produce crystalline insulin, the most important factor is to have a good raw material (namely, the fresh pancreas of slaughter-house animals) as well as the space and equipment indispensable for this type of production. The specialist directly concerned with production should have enough experience in this particular field. For this project's objective it was important also to learn the total quantity of insulin required for Burma.

The Central Research Organization, Rangoon

CRO, which had its origin in the State Industrial Research Institute established in 1948, reached its present level in staff and budget some fifteen years ago. The technical staff numbers approximately 200. About one-third are science graduates. Many hold post-graduate degrees from universities abroad. CRO is a multidisciplinary research institute with fourteen departments. Its laboratories are housed in twenty buildings on a 36-acre site in a suburb of Rangoon. They are fairly well equipped for most work of an applied scientific nature. CRO, under the supervision and control of the Ministry of Industry, operates through a Chairman assisted by an Advisory Board of CRO Research Directors as well as scientists and technologists from other institutes.

The production of insulin falls under the CRO's Pharmaceutical Department. This has five sections mostly concerned with the isolation of materials from their natural sources. The head of the Department has a doctor's degree in organic chemistry, and is Deputy Director of CRO. Seventeen individuals are employed in the Department. Five are specialists (four chemists and one botanist); seven, technicians; and five, laboratory attendants.

Work programme

To study the work protocols on CRO experiments to obtain crystalline insulin from beef pancreas

To learn about present CRO equipment and potentialities for insulin production

To process one or two batches of crystalline insulin from beef pancreas on the same scale as performed hitherto at CRO

To make proposals during the processing for improvement of the preparation method and on how to obtain crystalline insulin of greater purity and higher yield

To visit the Rangoon and Mandalay slaughter-houses to find out the number of animals slaughtered and the possibilities for collection of fresh beef pancreas for insulin production

To discover the facilities for the cleaning, rapid freezing, and transportation of frozen pancreas from the slaughter-houses to CRO

To visit Rangoon and Mandalay hospitals to ascertain the number of people suffering from diabetes mellitus in Burma and the percentage of diabetics needing insulin

To find out the quantity and cost of imported insulin injections in Burma

To discover an institution able and willing to develop methods for the biological assay of insulin

To discover an establishment able and willing to produce insulin injections from crystalline insulin

To make a list of equipment required for insulin production on a pilot-plant or smaller industrial scale at CRO

To propose the equipment for UNIDO investment in this project

SUMMARY

In Burma a possibility exists of producing insulin injections from domestic beef pancreas. Twice the quantity of insulin presently imported could be produced and this would meet the needs of two-thirds of the patients who lack this medicament. Some foreign exchange outlay would thereby be saved.

Arrangements have been made with local establishments for the biological assay of insulin, and for the production of plain and protamine zinc insulin injections.

The preparation of insulin injections from imported crystalline insulin is proposed until domestic crystalline insulin becomes available: thereby less foreign exchange will be expended than on the import of insulin injections.

An agreement has been reached with Rangoon and Mandalay slaughter-houses for the delivery of pancreas from all slaughtered cattle to the Central Research Organization (CRO) at Rangoon.

During the processing at CRO of two batches of insulin on a laboratory scale, suggestions were made and advice given for an improvement of the insulin production method. Crystals were obtained in one of the earlier experimental batches. Although many data indicate that it is insulin, this cannot be unambiguously proved without a biological test.

Procurement of equipment for increased production of insulin at CRO has been proposed to the Deputy Director. Although some equipment would be procured by CRO from its own funds, the majority would need to be financed. Effectuation would proceed in two stages, the first already authorized by UNIDO, the second still requiring authorization.

A competent chemist is directly engaged in insulin production, but training in a reputable insulin factory abroad would be necessary because of the special nature of the work.

A technical adviser on insulin should revisit Burma after a certain time to furnish fresh advice and recommendations.

I. PROJECT INVESTIGATION

A. CRO extraction activity

CRO laboratory-scale experiments for the production of insulin from beef pancreas were started at CRO in 1972. Batches of 2 to 5 kg of pancreas were processed from time to time. Until now 13 batches had been processed, 9 by the Romans method and 4 by the Jorpes method. 1/

Insulin is extracted from pancreas with acidic ethanol according to both methods. In the Romans method, the alcohol is removed by vacuum distillation and crude insulin is obtained from the remaining aqueous extract by saturation with sodium chloride. In the Jorpes method, insulin from the alcoholic extract is absorbed on alginic acid, then eluted, and from this eluate crude insulin is obtained in accordance with the Romans method. Further steps include the purification of crude insulin and, including crystallization, are generally the same in both methods.

At CRO there are no facilities for vacuum distillation as required by the Romans method, in which the temperature of the distillate should not exceed 20°C. The temperature of tap water for cooling at CRO is about 28°C. CRO has no cooling media for lower temperatures, but it produces alginic acid and has enough for insulin production purposes.

The Jorpes method gave better results. In one batch the crystals obtained could be clearly observed under the microscope (magnification=140 times) and had an identical melting point as insulin. The yield was 60 mg, i.e., about 1,400 International Units (I.U.) per kg of pancreas, assuming that the activity was 24 I.U. per mg of insulin. Unfortunately, the only test that will prove the activity of insulin is the biological assay, without which it is impossible to claim whether the crystals obtained are insulin or not. There is, however, no established method in Burma for the biological assay of insulin. No one has as yet been concerned with this problem. It has therefore been arranged to carry out the biological assay of the crystal sample assumed to be insulin at the pharmaceutical factory PLIVA, Zagreb, Yugoslavia.

1/ R. G. Romans, D. A. Scott and A. M. Fisher, Ind. Eng. Chem. 32 (1940) 908,910. E. Jorpes, V. Mutt and S. Rastgeldi, Acta Chem. Scand. 14 (1960), 1777 - 1780. Other procedures, including patented methods, are mostly modifications of these two methods.

B. CRO equipment and personnel

At CRO, insulin isolation is carried out in a standard laboratory equipped for routine extraction from plant sources and for organic synthesis. There are no air-conditioners. During the project the average temperature of the laboratory was 3[illegible]°C. There is a good vacuum pump for ordinary distillations and filtrations as well as a small-size, hand-driven meat grinder for pancreas.

A refrigerator at about 10°C, capacity 350 litres and with a small freezing compartment, is situated in a neighbouring laboratory. In another neighbouring laboratory is a freezer at -12°C, capacity about 500 litres, but it is not available to the Pharmaceutical Department.

The laboratory has a filtration centrifuge (basket centrifuge), 300 mm diameter, stainless steel, speed 5,000 rpm. It cannot, however, be used for insulin production because metals, especially iron in an acid medium, inhibit insulin activity.

A pH meter is essential to the production of insulin because at all stages pH must be accurately determined. The pH meter in the laboratory has been irreparably damaged. During the project a pH meter was borrowed from another department for a few days.

Experiments on insulin extraction are conducted, with the help of one laboratory attendant, by a chemist with 16 years' experience in the isolation of material from plant sources.

C. Experimentation

The complete procedure for obtaining crystalline insulin from two batches of beef pancreas was performed according to the Jorpès method. During the process advice was given to the chemist directly involved in the work. Twenty reprints and about a hundred abstracts (including patents) on the preparation of insulin from beef pancreas, were put at her disposal.

In the frequent discussions with CRO members engaged on the project every attempt was made to give as much information as possible.

D. Discussions with experts

For the success of the project it was necessary also to discuss various problems with experts from the following establishments:

Rangoon General Hospital
Mandalay General Hospital
Health Statistics Department
Central Medical Stores Depot (CMSD)
Department of Medical Research
Rangoon Municipal Corporation
Slaughter-houses in Rangoon and Mandalay
Burma Pharmaceutical Industry, Rangoon (BPI)

A list of participants and the dates of meetings are given in annex IV. Subsequently correspondence was exchanged to confirm the arrangements reached.

Diabetes mellitus incidence and insulin requirements

From these discussions it transpired that the percentage of Burmese suffering from diabetes mellitus is about the same as in Western countries, namely, 1 to 2% of the total population. As the population of Burma is about 30 million, this means that the average number of diabetics is about 450,000, 80% of whom are subject to a variant called tropical type diabetes.

The percentage of diabetics entirely dependent on insulin cannot be determined because no records are kept by the Department of Health. According to specialists from Rangoon General Hospital and Mandalay General Hospital, about three times as much insulin is required as is presently imported. They believe that it is enough to have only two types of insulin injections, i.e., plain and protamine zinc insulin. They regard work with insulin injections of other types, such as Lente, as unnecessary for their purposes.

Insulin imports into Burma

The following table was obtained regarding imports of insulin. Between 1965 and 1975 the average annual import of plain and protamine zinc insulin was 63,100 ml, 40 I.U. per ml ampules of 10 ml. That is 2,524,000 I.U. or 105.2 g of insulin (24 I.U. per mg) per year. The average price was K 3.16 per ampule, which represents an annual outlay of about K 20,000 in foreign exchange. The imported insulin is stored at 4°C at the Central Medical Stores Depot (CMSD).

Table. Burmese insulin imports, 1965-1975

Nomenclature	Department	1965-1966	1966-1967	1967-1968	1968-1969	1969-1970	1970-1971	1971-1972	1972-1973	1973-1974	1974-1975		Average per year
Insulin plain	CMSD	-	nil	10,000	-	2,880	700	73,890	49,970	-	35,000		21,500
40 I.U. (per cc)	AMSD	1,500	5,000	3,500	2,000	5,000	10,000	7,500	10,000	10,000	5,000		6,000
	TC 10	-	-	-	1,340	576	1,500	-	-	-	-		1,100
												Total	28,600
Insulin P/Z	CMSD	-	-	30,000	-	-	-	18,500	14,990	-	30,500		11,700
40 I.U. (per cc)	AMSD	1,500	5,000	2,000	-	3,500	5,000	5,000	7,500	6,000	3,000		3,850
	TC 10	-	-	-	-	-	-	-	4,500	-	-		4,500
												Total	20,000
Insulin zinc	CMSD	-	-	-	-	-	nil	-	-	-	-		-
40 I.U. (per cc)	AMSD	6,000	15,000	-	-	-	-	1,500	1,500	7,000	3,500		3,450
	TC 10	-	-	-	-	1,152	2,760	2,400	12,125	-	47,500		11,000
												Total	14,500
											Total		63,100 cc

Abbreviations

CMSD: Central Medical Stores Depot

AMSD: Army Medical Stores Department

TC 10: Trade Corporation 10

Biological assay of insulin

In the Department of Medical Research and at BPI there exist real prospects for the biological assay of insulin. Enough experimental animals, such as rabbits or mice, are maintained under suitable conditions (air-conditioned animal houses). Specialists from these establishments can successfully perform these assays and are interested in introducing this method. They were provided with some literature on the subject, two reprints regarding the various methods of biological assay as well as one on the biological standardization of insulin recognized by the United States Pharmacopoeia XVI and another used by PLIVA.

Preparation of insulin injections

At BPI there is a reasonable prospect for the preparation of insulin injections from crystalline insulin. The Injection Department has equipment for sterile filtration (Ssits filters), essential for the preparation of such injections. The staff, experienced in the production of sera and vaccine injections, have shown interest in the production of insulin injections. Methods for the preparation of plain and protamine zinc insulin injections were passed to BPI.

At the meeting with BPI representatives at CRO and according to a communication from the Deputy Director of CRO to BPI, it was suggested that, before CRO can produce insulin on a larger scale, BPI should produce insulin injections from imported materials, i.e., crystalline insulin and protamine. In this way the quality of the injections will be improved because it avoids the long transport by sea at high temperatures. Insulin injections should be kept at 2° to 4°C, because above this level soluble insulin is quickly destroyed. Crystalline insulin in the form of powder is more stable and can sustain temperatures up to 50°C. Moreover, since the price of imported crystalline insulin for the same quantity of injections will most likely prove to be lower than the price of the imported finished products (injections), foreign exchange will be saved. The import of crystalline insulin stands a fair chance of realization because BPI has foreign exchange at its disposal.

The organic chemist engaged at CRO in insulin production was given instruction, verbal and written, in the preparation of protamine (a basic element of protamine zinc insulin) from the spawn of mullet (mugil cephalus). No experiments were performed because no raw material was available.

E. Site for crystalline insulin production

It would seem more appropriate that initially the production of insulin from slaughter-house animals should be carried out at a pharmaceutical centre. For Burma it may, however, due to the absence of financial profit as well as the specific, time-consuming, and sensitive nature of the process, be better to produce insulin on a smaller industrial scale at CRO. The experts at CRO and BPI are of similar opinion. Experiments performed at BPI three years ago to obtain crystalline insulin from pig pancreas did not succeed and the idea of producing crystalline insulin on their own was abandoned.

It is advisable, because of the character of this work, for the biological standardization and preparation of insulin injections not to be carried out by the same establishment as produces insulin. This view came to be accepted by members of CRO during the course of the project.

F. Slaughter-house conditions

Insulin, as a medicament, should be prepared from only one kind of slaughter-house animal because the insulin from cattle, pigs, sheep, horses, and others differs in amino acids types. The difference is most pronounced between the insulins from cattle and pigs: they differ in as many as five amino acids. Today it is well known that cattle insulin is the most akin to human insulin. That is why in the treatment of diabetics with insulin prepared from beef pancreas there occur fewer cases of side-effects such as allergy and shock than in treatment with pig insulin. The highest frequency of side-effects has been observed in treatment with insulin prepared from the pancreas of assorted animals like beef and pigs. Work with pig pancreas is more difficult than with beef pancreas because there is always more fat. Beef pancreas is, therefore, chosen as the best raw material for crystalline insulin production.

Slaughter-houses and pancreas

Slaughter-houses play the most critical part in this project. The primary condition for good production of insulin is to have the raw material as fresh and clean as possible. It is necessary to ensure a sufficient quantity of pancreas from the slaughter-house or slaughter-houses. It must be neatly cleansed of fats, connective tissues and lymph nodes. After the cleaning, it

must be deep-frozen as early as possible and at the latest two hours after the slaughter. The best thing is to put it at -20°C in a deep freezer. Longer storage at room temperature will initiate the process of autolysis by the enzymes trypsin and chymotrypsin which are also present in the pancreas. Therefore, the amount of insulin in the pancreas will rapidly decrease.

From 1,000 to 3,000 I.U. of insulin are obtained from every kg of beef pancreas, depending upon the age of the animal. More insulin is obtained from young cattle. From animals older than nine years only about 1,000 I.U. per kg of pancreas can be obtained. In Burma, the slaughter of cattle younger than 13 years is forbidden. Consequently the quantity of insulin in the pancreas of these animals will not exceed 1,000 I.U. per kg.

The officials of the Rangoon and Mandalay slaughter-houses are quite willing to collect for CRO pancreas for insulin extraction. The problem is the freezing because the slaughter-houses have no cooling facilities at all, at CRO there is no deep-freezing facility readily available, and no possibility exists for transporting frozen pancreas from the slaughter-houses to CRO. In Rangoon the trip takes half an hour; from Mandalay it will take three to four days if sent down the Irrawaddy.

Domestic pancreas supplies

According to the information given by the Veterinary Officers of the Rangoon Municipal Corporation and from the Rangoon Slaughter-house, 25,000 to 30,000 cattle are slaughtered annually in Rangoon. Taking an average of 6 pancreases to 1 kg, the collection of 4,200 to 5,000 kg of beef pancreas means an average annual total of 4,600 kg. At K 1.84 per kg, this gives a yearly outlay of about K 8,450. If no more than 1,000 I.U. of crystalline insulin are obtained from 1 kg because of the animals' age, then 4,600,000 I.U. of insulin will be extracted annually. At 24 I.U. to 1 mg of crystalline insulin, 192 g could be produced per year from the whole supply of pancreas from Rangoon slaughter-houses.

According to data received from the Veterinary Officer of Mandalay Slaughter-house, some 6,800 cattle per year are slaughtered there. This is about 25% of the figure for Rangoon and amounts to some 1,150 kg pancreas annually. At K2 per kg at Mandalay, much the same price as at Rangoon, the cost would be

about K [illegible],300 per year. Calculated on the same basis as for Rangoon, the total pancreas supply from Mandalay Slaughter-house annually could produce 1,150,000 I.U. or 18 g of crystalline insulin.

In short, the total quantity of beef pancreas supplied by Rangoon and Mandalay slaughter-houses (4,600,000 + 1,150,000 = 5,750,000 I.U.) could produce 230 g (25 I.U. per mg) of crystalline insulin per year.

Domestic production prospects

Compared to the annual import of insulin into Burma, i.e. 2,540,000 I.U., this would be 2.26 times, or if only the Rangoon Slaughter-house is taken into consideration 1.81 times, more insulin.

Consequently, there exists a possibility for producing from domestic raw material twice the quantity of insulin now imported and reducing the present deficiency which Rangoon General Hospital experts estimate to be three times below requirement.

G. CRO production plant capacity

With 5,750 kg of pancreas from the Rangoon and Mandalay slaughter-houses at its disposal, CRO would process (on the assumption of 300 working days per year) some 15-20 kg daily. For that capacity a plant of smaller dimension, perhaps a bigger pilot plant to take production to the point at which crude insulin is obtained by saturation of the pancreas with sodium chloride, can be taken into consideration. Further steps, which include purification and crystallization, would be performed on a larger laboratory scale.

All raw materials needed for the production of crystalline insulin, except for a small quantity of Dowex-50 resin, are on hand. Most of them, like ethanol and alginic acid, can be recovered and used over and over again.

After thorough discussions with the Deputy Director, CRO, a list of the equipment and space necessary at CRO for such production was put into writing (annex V). CRO will procure from its own funds a part of these requirements, i.e., an air-conditioned production site, a cold room at 2° to 4°C including cooling machinery, two stirrers for about 100 litres of pancreatic extract each, a meat grinder, and several polyethylene tanks for pancreatic extracts.

II. CONCLUSIONS AND RECOMMENDATIONS

A. Domestic production of crystalline insulin

Domestic production of crystalline insulin will include the entire amount of pancreas to be obtained from the Rangoon and Mandalay slaughter-houses. The need is for thorough experience, more equipment, and better working conditions (annex V). The procedure for obtaining insulin, a lengthy process which requires careful, precise work, will in the near future be performed on a laboratory-scale and be expanded later to a smaller-scale plant. Increased production will gradually replace imports of crystalline insulin until these are wholly eliminated. Eventually production will amount to twice the quantity that is at present imported. Financial assistance will be necessary for the majority of equipment required.

Although some indirect gain will emerge from a reduction in foreign-exchange outlay, the project's success will not be of a profit-making nature. For this reason it is important that the largely government-sponsored CRO, rather than BPI, should undertake production. The social aspect is that not only diabetics who otherwise cannot survive for lack of insulin will be helped, but that domestic production will in time allow double the number of patients as at present to be treated.

The short- and long-term recommendations which follow here should be implemented concurrently.

B. Short-term recommendations

Biological assay of insulin

A method for biological assay of insulin should be established at CRO Department of Medical Research and at BPI.

BPI procurement of crystalline insulin

A minimum quantity of 100 g (24 I.U. per mg) of crystalline insulin should be imported annually by BPI for the manufacture of insulin injections. The same amount is presently imported into Burma. If possible, a larger quantity should be procured. This would in any case save foreign exchange because crystalline insulin would be less expensive than ready-made insulin injections.

Insulin injections

BPI should gain experience in the production of insulin injections so as to meet a national need.

C. Long-term recommendations

Equipment

Procurement of equipment falls into three categories:

(a) Purchase with existing UNIDO authorization;

(b) Purchase with outside assistance because of CRO inability to procure such equipment from its own resources;

(c) Purchase by CRO (annex VII).

Deep freezers and isolation boxes. Two deep freezers and two isolation boxes should be purchased at the same cost ($1,000) but in substitution for the rubberized centrifugal basket-head authorized by UNIDO and included in the project budget (annex III). Technical data and cost are given in annex VIII. UNIDO Requisition for Equipment Form No. 1 has been completed.

Meter and centrifuges. Additional financial assistance will be necessary for the purchase of one pH meter, three types of centrifuges, one deep freezer and six isolation boxes. The total cost will be $17,980. Technical data and cost are given in annex IX. UNIDO Requisition for Equipment Form No. 2 has been completed.

Staff

Increases. For the efficient working of larger-scale insulin production one more chemist or chemical engineer, three technicians, and six to eight laboratory personnel will be needed in addition to the chemist and laboratory attendant at present employed.

Training. Insulin production is so specific and sensitive that it will be very usuallyfor a CRO chemist to undergo training outside Burma for a period not exceeding six months. This will enable the trainee to become familiar with all operations involved and to pass this knowledge to colleagues who will be working on insulin injections and biological assay. The approximate cost will be $6,000. The chemist engaged in insulin extraction at CRO has some working experience and 16 years' experience in the isolation of material form natural sources.

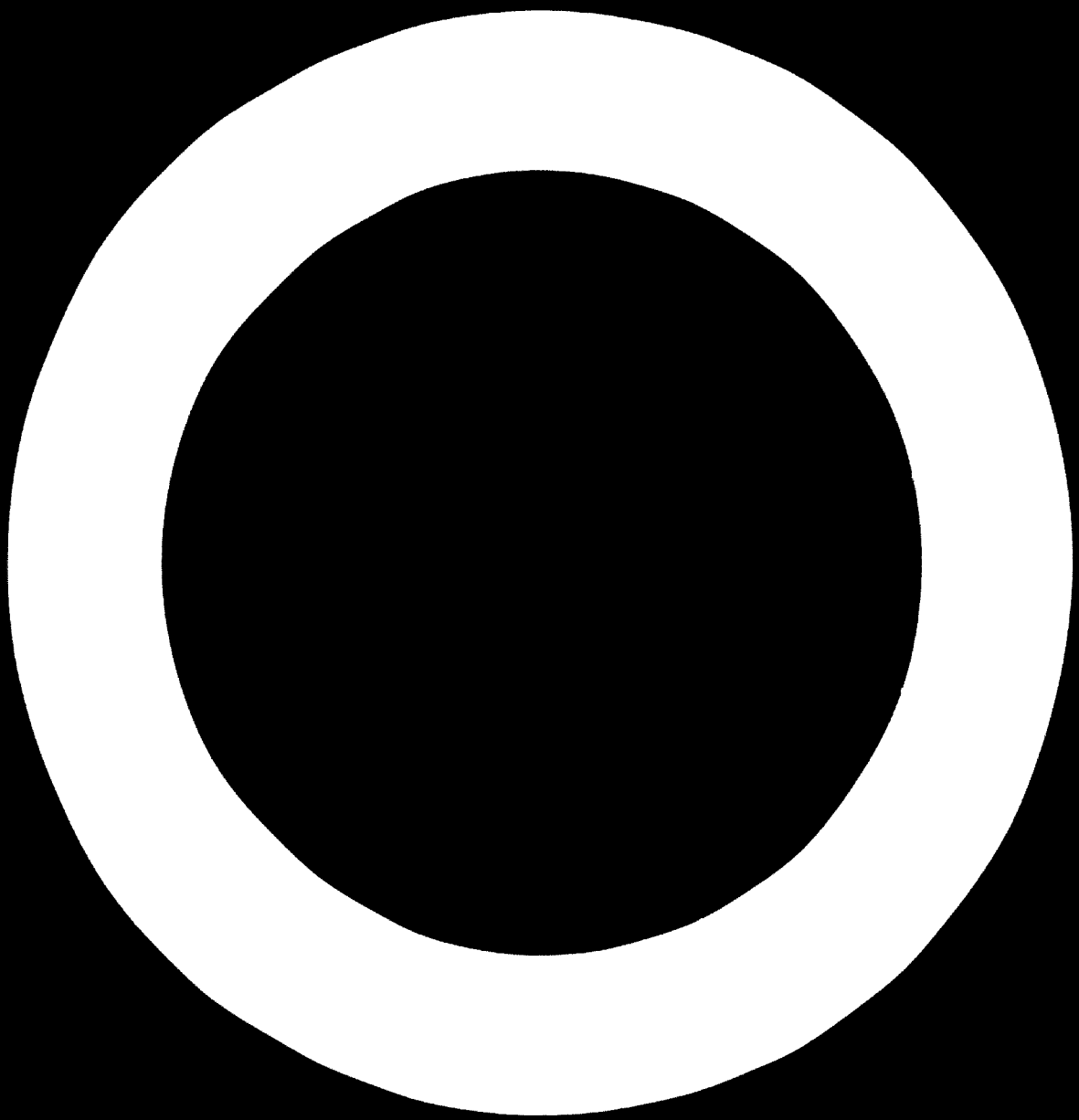
The value to the trainee of work in a factory with long experience of insulin production will be the acquaintance with all sources of trouble arising during the production process. Such factories exist in many countries. The PLIVA factory at Zagreb, Yugoslavia, which began like the CRO as a research institute, has been producing insulin for 35 years and might be a suitable site for such trainee specialization.

Study of the complete procedure for insulin production, with special emphasis on crystallization and regeneration of mother-liquors during the different stages of production, as well as study of protamine production from mullet spawn would take about four months. For observation of plain and protamine zinc insulin injections some two weeks should be allowed. The method of biological assay as practised with rabbits should be studied for a month and a half.

Future developments

It will be useful, after the equipment has been procured and the recommendations have been implemented, for an expert to visit Burma to evaluate the work done, discuss with CRO specialists the new developments, and give further instruction as well as recommendations on how work should be undertaken in the conditions likely to prevail in future.

Such an expert should hold, if possible, a doctorate in biochemistry and possess long practical experience in the field of insulin production.



Annex I

JOB DESCRIPTION

POST TITLE	Technical Adviser for Insulin Extraction
DURATION	Two months
DATE REQUIRED	As soon as possible
DUTY STATION	Rangoon
PURPOSE OF PROJECT	To assist the Central Research Organization of Burma in the laboratory scale extraction of insulin from beef pancreas and scaling up of the process.
DUTIES	Specifically, the expert will be expected to: 1. supervise the work at the Central Research Organization on extraction of insulin from beef pancreas to achieve higher yields and purity; 2. train the staff in the techniques of obtaining insulin in a crystalline form; 3. advise on matters relating to the scaling up process; 4. prepare a list of equipment which will be required to produce insulin solutions on a small scale for clinical use; 5. ascertain the optimal system of packaging of insulin based on the possibility of local packaging products.
QUALIFICATIONS	Biochemist or pharmacist with wide experience in the extraction and purification of insulin from beef pancreas.
LANGUAGE	English
BACKGROUND INFORMATION	The Central Research Organization of Burma has made preliminary studies on the technical feasibility on the manufacture of insulin by extraction from beef pancreas because of difficulties encountered in the import and storage of insulin preparations for clinical use. From the abattoirs around Rangoon 100 kg of beef pancreas for insulin extraction could be collected per day. The Central Research Organization of Burma has difficulties in getting insulin in the form of crystalline to monitor the course of extraction due to the lack of biological assay facilities. The Central Research Organization studied on laboratory scale extraction is based on the methods of Romans, Scott and Fisher, Ind. Eng. Chem. 32 (1940), 908 and those of Jorpes, Mutt and Rastgeldi, Acta. Chem. Scan. 14 (1960), 1777.

Annex II

PROJECT PERSONNEL

UNIDO consultant

Oleg Scedrov, technical adviser, 22 March - 10 May 1975.

Counterpart personnel

Dr. Mehm Thet San, Director General, CRO, organic chemist: 24 March - 12 April 1975.

Dr. Maung Maung Gale, Deputy Director, CRO, organic chemist: 24 March - 10 May 1975.

Mrs. Tin Tin Ohn, Research Officer, CRO, organic chemist: 24 March - 10 May 1975.

Annex III

BUDGET DATA

Project budget (according to project data sheet)

Components:

Expert in extraction of insulin (for 2 months)		$ 5,000
Equipment not available in the country: a rubberized centrifugal basket-head for filtration of insulin solutions to be attached to existing centrifuge		$ 1,000
	Total	$ 6,000

CRO Budgets

1972-1974

Salaries		K 46,000
Chemicals and supplies		K 6,000
	Total	K 52,000

1975-1978

Salaries		K 100,000
Chemicals, supplies, equipment, and building materials		K 60,000
	Total	K 160,000
Total 1972-1978		K 212,000

Annex IV

MEETINGS WITH EXPERTS FROM OTHER ESTABLISHMENTS

Rangoon General Hospital, 11 April 1975

Dr. Maung Maung Aye, medical superintendant
Prof. R. Ba Pe, internist
Dr. Daw Hnin Yee, internist
Dr. Khin Maung Win, internist
Dr. Maung Ko, clinical pathologist
Dr. U E, medical stores officer
(Received the dissertation of Than Yin Mar, The Clinical Profile of Diabetes Mellitus in Rangoon General Hospital, December 1974, 51 p.)

Mandalay General Hospital, 9 April 1975

Dr. U Ko Ko, medical superintendant
Dr. Tin Aung Swe, specialist for tropical diseases

Health Statistics Department, 23 April 1975

Dr. Daw Yin Mya, Assistant Director

Central Medical Stores Depot, 21 April 1975

Dr. Saw Lwin, Deputy Director of Health

Rangoon Slaughter-house, 1 April and 19 April 1975

U Hla, veterinary officer, Head, Slaughter-house

Mandalay Slaughter-house, 9 April 1975

U Thwin, veterinary officer, Head, Slaughter-house

Department of Medical Research, 1 April 1975

Dr. Mya Tu, Director General
Dr. Kywe Thein, Deputy Director
Dr. S.J. Tha, pharmacologist
U Hla Pe, Head of Biochemistry Research Division
U Chit Maung, senior research officer, Pharmaceutical Research Division

CRO, 22 April 1975 with representatives of BPI:

U Hla Thaung, Pharmaceutical Director
Dr. Ko Ko Gyi, bacteriologist
U Soe Hlaing, quality control chemist
U Maung Maung Mya, biologist

Discussion, 5 April 1975 U Ba Ngwe, Veterinary Officer, Rangoon Municipal Corporation

Annex V

CRO EQUIPMENT AND SPACE REQUIREMENTS FOR CRYSTALLINE INSULIN PRODUCTION

Deep freezers

325 litres (11.5 cu ft), storage temperature -22°C with ambient temperature to 42°C, with wire baskets. For 220 V, 50 c/s, 180 W (1/4 hp), Gross 180 kg, shipping volume 1.45cu/ m.Karl Kolb 1974 Catalogue, No. 231-260

Karl Kolb
D-6079 Buchschlag-Frankfurt
P.O. Box 100
Federal Republic of Germany

Price: $500

One item

or: 350 litres, storage temperature - 18°C, with ambient temperature up to 42°C. For 220 V, 50 c/s, 1/5 hp. Over-all dimension 74 x 66 x 180 cm high Gallenkamp 18th Catalogue, No.RJ-390

Gallenkamp
Technical House
P.O. Box 290
Christopher Street
London EC2P 2ER
England

Price: $550

One item

225 litres (7.9 cu ft), storage temperature -22°C, with ambient temperature up to 42°C, with wire baskets. For 220 V, 50 c/s. 130 W (1/6 hp). Gross 100 kg, shipping volume 1.2 cu/m. Karl Kolb 1974 Catalogue, No. 231-280

Price: $440

Two items

Total $880

...r: 345 litres, storage temperature -20°C, with ambient temperature up to 42°C. For 220 V, 50 c/s 1/5 hp
Over-all dimensions 63 x 97 x 85 cm high
Gallenkamp 1974 Catalogue, No.RJ-975

Price: £ 300

Two items

Total £ 600

Box specially isolated with polystyrene, so-called "week-end refrigerator for car", about 70 litres capacity (for transport of frozen pancreatic glands from slaughter-house to CRO)

Price: about £ 30

Eight items:

Total £ 240

Refrigerated room, size: 3.50 x 2.00 x 1.80 m, at 2°C to 4°C, isolated, including cooling machinery
Cooling machinery available at CRO

Price: K 5,000

One item

A room (12 x [illegible] x 3 m) for production of insulin from pancreatic glands, with air-conditioning equipment available at CRO

One item

Meat grinder (a common butcher size), full length 400 to 500 mm, with two knives and two plates, and with electric motor (220 V, 50 c/s), including spare knives and plates

Price: about £ 100

One item

Cylindrical or conical polyethylene tanks (for pancreatic extracts)
Karl Kolb 1974 Catalogue

Capacity (litres)	Catalogue No.	Unit price (£)	Number of items	Total cost (£)
200	580-083	84.50	2	169.00
110	580-052	29.00	6	174.00
85	580-062	11.70	3	35.10
60	580-061	10.60	4	42.40
40	580-060	8.65	4	34.60
25	580-080	19.80	3	59.40
			22	514.50

Stirrer with 25 to 30 rpm, for 100-litre mixtures, with electric motor (220 V, 50 c/s)
(... has the electric motor)

Price: K 250

Two items

Total K 500

pH-meter Beckman Zeromatic SS-3, 9603 with spare electrodes.
pH range 0 to 14 pH, relative accuracy ± 0.05 pH, temperature 0°C to 100°C, for 220 V, 50 c/s, 15 W

Price: $900

One item

Basket centrifuge, diameter 600 to 700 mm, speed 5,000 rpm, inside parts covered with rubber or plastic lacquer to be resistant to 70 to 80% ethanol at pH 2 to 5 adjusted with hydrochloric acid or phosphoric acid

Gebr. Heine Zentrifugenfabrik
P.O. Box 360
Viersen (Rhld)
Federal Republic of Germany

Price: about $3,500

One item

Centrifuge-type Sharples, high speed, 30,000 to 50,000 rpm, with continuous flow, stainless steel resistant to pH 7 to 8, adjusted with ammonia, for 220 V, 50 c/s

Alfa Laval AB
Postfach
S-147 00 Tumba
Sweden

or: Cepa,
Carp Padberg Zentrifugenbau GmbH
763 Lahr/Schwarzwald
Federal Republic of Germany

Price: about $3,000

One item

Refrigerated centrifuge type K-70, for 380 V, 50 c/s, 3.5 kVA, speed 5,500 rpm, with attachment for high speed 20,000 rpm, minimal temperature -10°C, maximal capacity 4 x 1250 ml, including all accessories

Jurgens 1974 Catalogue, Nos. 101100 to 101194

H. Jurgens and Co.
P.O. Box [illegible]
Jurgens House
Lahrstrasse 70
Bremen
Federal Republic of Germany

Price: about $10,000

One item

r:

Large refrigerated laboratory centrifuge, Model Junior IV KS, for 380 V, 50 c/s, 4,800 W, speed 6,000 rpm, with high speed attachment 20,000 rpm, temperature -20°C to + 35°C, maximal capacity: 4 x 1000 ml, including all accessories

Karl Kolb 1974 Catalogue, Nos. 197750 to 197800

Price: about $12,000

One item

Grand Total $19,034.50 and K 5,500

Annex VI

EQUIPMENT SUBSTITUTION

The project budget (annex III) authorised the purchase of a rubberised centrifugal basket-head to be attached to the existing centrifuge for the filtration of insulin solutions. This type of centrifuge is needed in the first stage of insulin production.

Pancreas is extracted in acid ethanol and filtered through gauze. From the remainder of the pancreas, after a second extraction, about 5% to maximum 10% of extract may be obtained after centrifugation in such a centrifuge. On the other hand, storage of pancreas after slaughter without rapid freezing leads to a greater proportion of insulin destruction than that which is obtained by centrifugation. After standing 24 hours at Rangoon room-temperature, which was about 37°C during the project, there would probably not be any insulin left in the pancreas. Transportation in ice would not solve the problem, because the autolysis of pancreas proceeds at 0°C, although at a slower rate (according to van't Hoff rule), until the pancreas is completely frozen. Only then will all autolytic processes stop. It may also happen that the pancreas cannot be extracted on the same day at CRO. In that case too, efficient freezing of the pancreas is necessary.

The frozen pancreas should not thaw during transportation. That is why it is necessary to have a special isolation box. This can be easily procured. There are portable coolers for week-end use which can be put in a car and could be successfully used for the transport of frozen pancreas.

The basket centrifuge available at CRO is moreover too small in capacity for use in insulin production at the full capacity which is planned. It is not enough to have only a rubberised basket. All the inside parts of the centrifuge which come into contact with the extract must be similarly protected. Acid alcohol extract of pancreas which has been adjusted to pH 2-5 with either hydrochloric acid or phosphoric acid will affect every type of stainless steel. It is well known that iron ions are very strong inhibitors of insulin and destroy it quickly. The very good plastic lacquer covers are today available which are cheaper than a rubberised centrifuge.

In view of these considerations it is proposed, in place of a rubberized centrifugal basket-head, to buy for the same cost equipment for the freezing and transportation of pancreas, the first and most important requirement for insulin production. Only raw material of good quality can furnish a product of high yields and purity. In fresh (which here means frozen) pancreas, there will be less destroyed elements than in unfrozen and therefore production will be able to be carried out more easily.

Annex VII

CRO EQUIPMENT PURCHASE

A. Analysis

CRO, from their own funds, will ensure the following needs as soon as possible:

(a) A small cold room (2° to 4°C) to be used for the purpose of storing extracts and solutions during certain stages of production. When working to full capacity, the first extract will be about 100 litres and the usual laboratory refrigerator will not be sufficient. CRO has at its disposal the cooling machinery necessary for such a cold room, but will have to see to its isolation;

(b) Two stirrers with 25 to 30 rpm for first and second extraction of pancreas. The electric motor which will drive the stirrers is available;

(c) A meat grinder from their own foreign exchange. This will not be a big expense and it is important to have an efficient meat grinder for larger-scale production;

(d) Twenty-two polyethylene tanks will be bought by CRO from their own foreign exchange. Tanks for insulin extracts can be of a hard plastic such as polyethylene which is not too expensive and very useful because it is light.

B. Technical data and cost

Cold room (2.50 x 2.00 x 1.80 m) at 2° to 4°C, including cooling machinery. Cooling machinery available at CRO	
Price for isolated room	K 5,000
One item	
Stirrer with 25 to 30 rpm, for 100 litres mixtures, with electric motor (CRO has the electric motor)	
Price for a stirrer K 250	
Two items	
Total	K 500
Grand total for domestic expenses	K 5,500
Meat grinder, full length 400 to 500 mm, with two knives and two plates with electric motor	
Price	$100
One item	
Tanks, polyethylene, 22 various items, total price	$514.50
Grand total for the procurement from abroad in foreign exchange	$614.50

Annex VIII

BASIC EQUIPMENT

A. Analysis

It is proposed to purchase two deep freezers at -20°C, one with about 230 litres capacity for the slaughter-house in Rangoon and with about 330 litres capacity for CRO. This temperature is necessary for rapid and efficient freezing of pancreas and is the usual method.

The Rangoon Slaughter-house has the space for installation and supply of electricity for such a deep freezer. This size is necessary in case pancreas has to be collected for several days at the slaughter-house.

CRO must have a bigger deep freezer for the storage of pancreas for several days in case of a possible production stop (for example, holidays) and also in order to process in larger batches.

Two isolation boxes, with about 20 litres capacity, are needed for transporting pancreas. The size will be sufficient for the daily transport of pancreas, i.e. 15 to 20 kg.

The purchase of this equipment would fulfil the primary condition for efficient insulin production. The deep freezer at CRO will be sufficiently large for the planned maximum capacity of pancreas from Rangoon and Mandalay slaughter-houses.

B. Technical data and cost

Deep freezer about 330 litres, storage temperature -22°C, ambient temperature up to 42°C	
Cost about	$500
Deep freezer about 230 litres, storage temperature -22°C, ambient temperature up to 42°C	
Cost about	$440
2 isolation boxes, about 20 litres capacity	
Cost about $ 30 each	$60
Total	$1,000

Annex IX

ACCESSORY EQUIPMENT

A. Analysis

pH meter

A pH meter is essential during the whole production of insulin. At every stage the pH must be strictly defined, sometimes with the accuracy of pH 0.05. Without this instrument one cannot even think of insulin production. Even crystallization is effected under pH meter supervision, with constant immersion of electrodes in the insulin solution. The existing instrument at the CRO Pharmaceutical Department is definitely out of order. The purchase of a new instrument is urgently necessary. CRO cannot procure it from its own funds.

Filtration (basket) centrifuge

A filtration or so-called basket centrifuge, diameter 600 to 700 mm, twice as big as the one at CRO, is necessary for filtration of the pancreas remainder after acid alcoholic extraction. It must be emphasized again that iron, even in a very minute quantity, will inhibit insulin. In an acid medium a small quantity of iron from the equipment will however always dissolve. The need for such a centrifuge has been explained (annex VI). Increased insulin production requires such a centrifuge because it will increase the insulin yield by 5 to 10%, while not so important on laboratory-scale production, it will later be of great significance.

Sharples centrifuge

Another centrifuge is necessary for the increased production of insulin. After pancreas extraction with acid alcohol, the extract is made alkaline with ammonia to pH 7.8–8. In this way many other proteins and their hydrolysates are precipitated. This precipitate must be quickly and efficiently removed. Insulin extract should not stand in an alkaline medium for more than 4 hours at room-temperature because impairment takes place. After removal of the precipitate, the extract should be immediately acidified. On a laboratory scale it can be filtered through large funnels with filter-paper. On a plant scale this cannot be done because it would take too long. It must therefore be centrifuged in a high-speed, continuous-flow centrifuge at about 30,000 to 50,000 rpm. This is the ordinary Sharples type centrifuge.

Refrigerated centrifuge

A large laboratory-type refrigerated centrifuge, with complete accessories, will also be required. In many stages of insulin preparation, beginning with

sodium chloride saturation and including crystallization which will be carried out at CRO to maximum capacity on a larger laboratory scale, the precipitate must be removed from the solution. Usually this involves time-consuming and tedious filtrations. There is always a loss of more or less precipitate and filtrate, meaning loss of insulin which becomes more significant on a larger scale. This is avoided by centrifugation and saves a lot of working time. A refrigerated centrifuge is needed to avoid heating during centrifugation because, especially at higher speeds, such spells of heating will always impair a part of the insulin present. CRO cannot purchase a refrigerated centrifuge from their own funds.

Deep freezer and isolation boxes for Mandalay

After all the pancreas from Rangoon slaughter-houses has been processed, that from Mandalay slaughter-houses should follow. For that purpose another deep freezer, with 230 litres capacity, should be purchased. There is space for installation and requisite electricity supply at Manaalay slaughter-houses.

For transportation of frozen pancreas from Mandalay to CRO six isolation boxes will be needed. The number is larger than for Rangoon because of longer transportation time. As a maximum of 4 kg pancreas will be collected per day, it will be best to transport every 5 to 10 days. For each transport 2 to 3 isolation boxes will be required.

B. Technical data and cost

pH Meter, Beckman Zeromatic, range 0 to 14 pH, relative accuracy ± 0.05 pH

Cost $900

Basket centrifuge, diameter 600 to 700 mm, speed 5,000 rpm, inside parts covered with rubber or plastic lacquer

Cost about $3,500

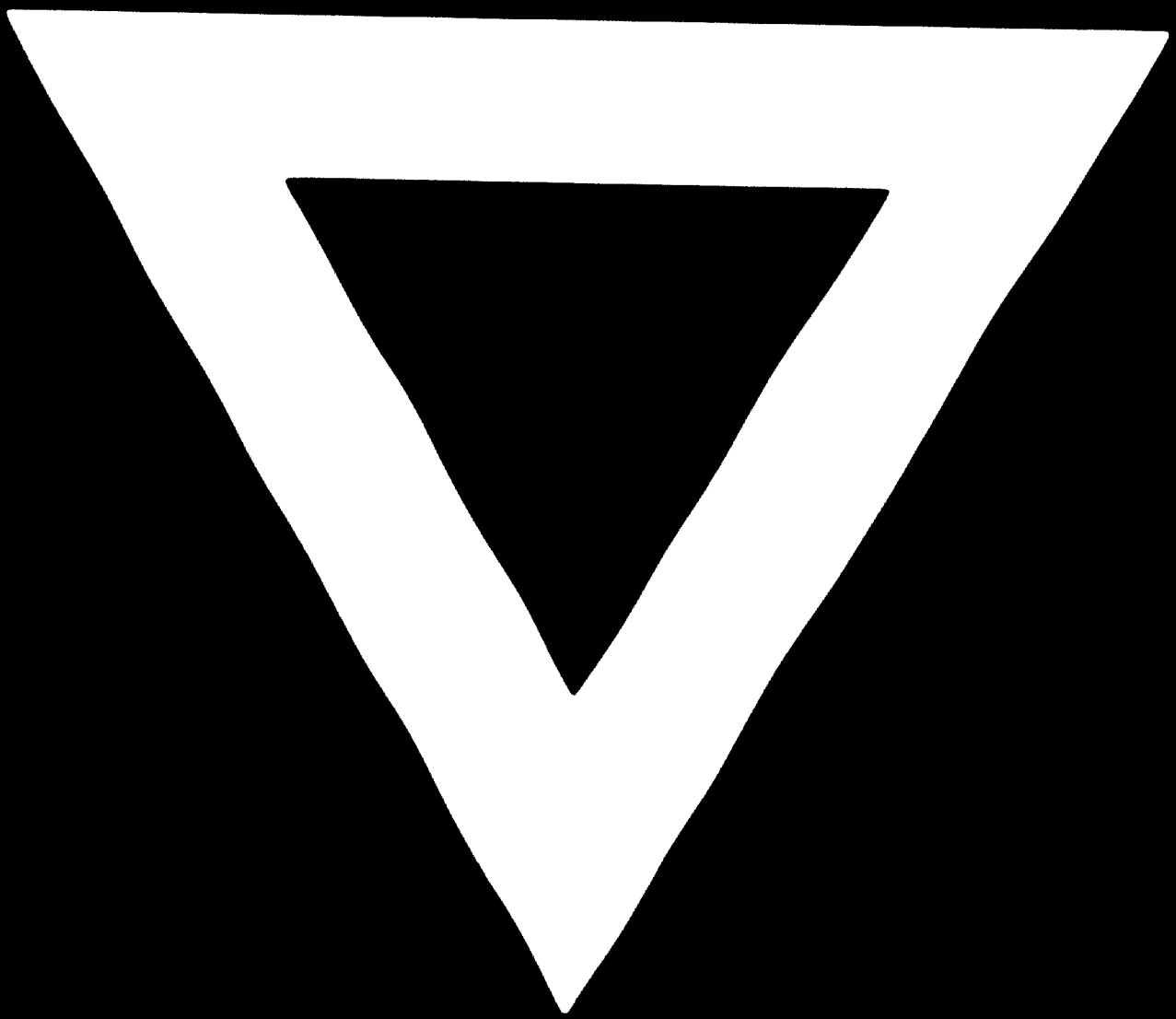
Sharples centrifuge, 30,000 to 50,000 rpm, with oontinuous flow

Cost about $3,000

Refrigerated centrifuge, large laboratory type, speed 5,000 to 5,500 rpm, with attachment for high speed 20,000 to 25,000 rpm, minimal temperature -12° to -20°C, maximal capcity 4 x 1,250 ml or 6 x 1,000 ml, including all accessories

Cost about $10,000

Deep Freezer, about 230 litres, storage temperature -22°C, ambient temperature up to 42°C	
Cost about	$400
	$17,800
6 isolation boxes about 20 litres capacity, for transportation of frozen pancreas	
Cost about $30 each	$180
Total	$17,980



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