OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

FAIR USE POLICY

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

17473

Distr.
RESTRICTED

IO/R.97
10 April 1989

UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

ORIGINAL: ENGLISH

ESTABLISHMENT OF A PILOT DEMONSTRATION PLANT FOR THE PRODUCTION OF VACCINES FOR AFRICA

XA/RAF/88/666

Technical report: Establishment of a quality control laboratory for the production of tetanus vaccines at Lanavet*

Prepared for the Government of Cameroon
by the United Nations Industrial Development Organization

Based on the work of L. Hegedüs
UNIDO Consultant

Backstopping Officer: Dr. Zoltan Csizer, Chemical Industries Branch

27

* This document has not been edited.

ACKNOWLEDGEMENTS

The consultant wish to acknowledge the full courtesy of the officials ot the Goverment and Institutions in Cameroon for their kind assistance and support in providing the necessary cooperation to undertake this study.

I am particulary grateful to

Dr. Ghogomu Nongho Amida /MD/ Director of Preventive Medicine, Ministry of Health
Dr. Maikano Abdoulaye, General Director, LANAVET
Dr. Abdoulkadiri Souley, Adjoint Director, LANAVET
Dr. C. Dobo Beye, Director, ONAPHARM
Dr. Abel Bollo Epee, Pharmacist, ONAPHARM
Mr. Konare, M. SIDFA
Mr. Coppini, P. President Representative of UNDP
for their wholehearted help and hospitality during my mission.

INTRODUCTION.

A mission was organized by UNIDO to assist the Government of Cameroon in the establishment of a pilot demonstration plant for the production and quality control of human vaccines at LANAVET, Garoua.

The consultants of vaccine production /J.Zsidai and L.Gy.Hegedüs/ fulfilled their mission in September and October 1988, while that for the quality control of vaccines /L.Hegedüs/ in November and December 1988.

The technical report concerning the production and other problems of human vaccines was published on 15 November 1988.
The present report, dealing with the possibilities of the establishment of the quality control system at local /in LANAVET/ and national level, represents an integral part of the report of 15 November 1988 /Chapter VII., Quality Control, page 79./.
For this reason, general paragraphs, as explanatory notes, abbreviations, project background and history, project implementation schedules and others are not repeated in this report.

VII. QUALITY CONTROL. /Chapter of the Report published on 15 November 1988/.

A. General Considerations.

Regulated by a number of WHO requirements, all vaccine production must be based on a reliable quality control system. Quality control is imperative, i.e. no vaccines without control should ever be used.

Each batch of a vaccine product has to be tested in the control laboratory in conformity with WHO requirements. Such tests include especially the control of safety, potency, sterility and identity of the vaccines. Special tests and rules exist for almost every vaccine, but here only those for the quality control of Tetanus Toxoid, Diphtheria-Tetanus and Diphtheria-Pertussis-Tetanus will be detailed. The quality control of other vaccines, included in the EPI as those against Tuberculosis, Measles and Poliomyelitis, will be described shortly.

An independent quality control unit is the basis for any development in the field of vaccine production. To achieve effective control of quality, the following rules might be applied:

a/ Adequate facilities and staff should be available for sampling, inspection and testing of starting materials, packaging materials, intermediate, bulk and finished products and where appropriate, for determining environmental quality.

b/ samples of starting materials, packaging materials, intermediate products, bulk and finished products should only be taken by personnel and using methods approved by the person responsible for quality control.

c/ Results of the inspection and testing of materials and intermediate bulk or finished products should be supervised and released by a designated person /chief in charge of quality control/ before products are given out for sale or supply on the producer's level.

d/ Sufficient reference samples of starting materials and products should be retained /the latter, where possible, in the final package/ to permit future examination if necessary.

The following points are of special importance in the control and release of human vaccines:

1. The vaccine producer has to perform every test required by the WHO requirements and/or national regulations in the country where the vaccine is produced. The results of all tests have to be submitted to the National Control Authority if existent.

2. The National Control Authority in the country, where the vaccine is produced, has to approve the vaccine batches. This authority could prescribe to perform all or certain tests in its own laboratory /National Control Laboratory if available/ and/or may approve the batches on the data submitted in the producer's protocols.

3. There might be several reasons to retest certain batches of vaccines, locally produced or imported, in the country where they are used. Examples for such reasons will be detailed in the chapter dealing with the functions of the National Control Laboratory.

4. In the most countries, vaccines for human use must be registered by governmental authorities before their administration for vaccination purposes.

5. For testing the potency of most vaccines, standard or reference preparations with a defined potency /usually expressed in International Units/ are essential. Such preparations have been established by the WHO Expert Committee on Biological Standardization and are available by request to National Control Authorities. At a later stage, in many countries national reference preparations were established, their potency being expressed in International Units on the basis of comparative tests.

Regarding the quality control of human vaccines, the WHO requirements were generally accepted all-over the world. In some countries, national regulations have been formulated and been made compulsory by law, but as a rule, they are indentical with or very similar to the WHO requirements and practically are never incompatible with them.

B. Quality Control of Tetanus Toxoid, Diphtheria-Tetanus and Diphtheria-Pertussis-Tetanus Vaccines.

B.1. Glossary of terms.

Lf= Limes flocculans: the amount of toxin or toxoid which when mixed with 1 International Unit of antitoxin gives a Ramon flocculation in the shortest time.

Lf/mg N= flocculation units per milligram of total nitrogen as determined by the Kjeldahl method.

Lf/mg PN= flocculation units per milligram of protein nitrogen/PN/ which is usually determined by the precipitation of the proteins with trichloroacetic acid.

I.U.= International Unit is the specific activity of a stated amount of the International Standard as defined by the WHO Expert Committee on Biological Standardization.

L+ = the minimum amount of toxin which when combined with 1 I.U. of antitoxin kills an animal of defined weight in four days /this value is dependent upon mice or guinea pigs are used/.

Ld 50 = the amount of toxin that kills 50% of a group of animals within four days /this value differs for different animal species/.

M.L.D. = Minimal Lethal Dose, the amount of toxin which kills animals within four days /this value differs for different animal species/.

A.B.V. = Antitoxin Binding Value, a value which defines the toxin plus toxoid in a mixture /determined in animals/.

ED 50 = the dose of a vaccine which protects 50 % of the immunized animals against a challenge dose of virulent bacteria or toxin.

Single harvest: the toxic filtrate obtained from one batch of cultures inoculated, harvested and processed together.

Bulk purified toxoid: the processed purified material prepared from either a single harvest or a pool of a number of single harvests. It is the material from which the final bulk is prepared.

Final bulk: the final toxoid present in a single container from which the final containers are filled either directly or through intermediate container/s/.

Final lot or filling lot: a collection of sealed final containers that is homogenous respecting the risk of contamination during filling. The filling must be carried out in one working session.

B.2. Control of starting raw materials.

In general, tests on all materials used in production are required if applicable in the producer's facilities, although the testing of some chemicals can be done on the basis of identity and inspection of analytical specification provided by the suppliers.

B.2.1. Aluminium-phosphate gel /$AlPO_4$/.

Determination /summarized/.
Reagents:
0,05 M KOMPLEXON III. solution
0,05 M $Cu/NH_3/_4SO_4$ solution
2n NaOH solution
R H_2SO_4 solution
Murexid indicator

The gel or the gel containing solution /i.e. vaccine/ must be homogenized by shaking. To 1,0 ml of the gel 50 ml distilled water, 5,0 ml 2 n NaOH and 10,0 ml Komplexon III solution should be given. After boiling and cooling, the mixture has to be neutralized by addition of R H_2SO_4. The superabundance of the Komplexon III. will be titrated by means of 0,05 M cuprumtetramidsulphate solution by using Murexid as indicator. The deep blue colour of the mixture will become vivid green at the finishing point of the titration.
1,0 ml of 0,05 M Komplexon III. solution equals 6,097 mg $AlPO_4$ or 1,349 mg Aluminium.

B.2.2. Distilled water.

To be tested for: physical properties, pH, purity /lead and heavy metals, calcium, sulphate, chlorid, nitrit, nitrat, NH_3, reducing substances/

B.3. Filling and packaging materials.

B.3.1. Ampoules, vials.

Physical properties, measurements, permeability of light, chemical tests, determination of hydrolytic class, absence of heavy metals.

B.3.2. Rubber stoppers.

Physical properties, measurements, chemical tests /heavy metals, chlorid, sulphid, NH_3, zinc, absorption of ultraviolet light, reducing substances/

B.3.3. Cartons, boxes, leaflets.

/Quality, measurement, text/.

B.4. Tetanus Toxoid Vaccine Adsorbed.

As the production of this vaccine will be introduced in LANAVET from starting materials, quality control requirements and methods will be given more detailed.

B.4.1. Control of source materials.

B.4.1.1. Strains of Clostridium tetani.
Strains of C. tetani used in preparing tetanus toxoid shall be identified by a record of their history and all of tests made periodically for verification of strain characters.

B.4.1.2. Seed lot system.
The production of tetanus toxin shall be based on a seed lot system. Cultures of the working seed shall have the same characteristics as the cultures of the strain from which the parent seed lot was derived. The purity of each seed culture should be checked by the examination of Gram stained smears and only those cultures found to be pure are used for the inoculation of the medium.

B.4.1.3. Culture medium for production of toxin /Fisek-Müller-Miller/.

B.4.1.3.1. Test for sterility: 2x50 ml thioglycolate and 2x50 ml Sabouraud media are to be inoculated. Samples of media have to be kept at 37^{O} until completion of the fermentation.

B.4.1.3.2. Physical properties: yellow-brownish liquid, free from turbidity.

B.4.1.3.3. pH value: 7,2-7,3 when measured by electric pH meter.

B.4.1.3.4. Alpha-amino- N content.
Requirement: 1,3 mg/ml at least
Determination /summarized/.
From the 1:2,5 dilution of FMM medium 5,0 ml has to be given to 5,0 ml $CuPO_4$ reagent. 10 minutes later, the suspension solution should be filtered and measured by spectrophotometer at a wavelength of 625 nm against the control solution, containing of aa $CuPO_4$ and distilled water.

B.4.1.3.5. Glucose-content.

Requirement: 0,9-1,1 g/ 100 ml

Determination /summarized/.

From the 1:100 dilution of FMM medium 1,0 ml has to be given to 1,0 ml physiological saline solution. To this mixture, placed in ice-water bath, 4,0 ml of 0,2 % antron containing H_2SO_4 solution will be added while shaking. After treatment by 100 C and ice-water bath, spectrophotometric measure has to be performed at a wavelenght of 625 nm against the control solution, containing 1,0 ml saline and the antron reagent.

B.4.1.3.6. NaCl-content.

Requirement: 0,3-0,6 mg/ml

From the 1:5 dilution of FMM medium 10,0 ml should be given to 10,0 ml R-HNO_3. To this mixture, 10,0 ml of 0,1 n $AgNO_3$ and 0,5 g kalium-nitrate have to be given. After boiling for 3-4 minutes, to the cooled mixture 0,1 n kaliumrodanid should be given.

Indicator: 1,0 ml Fe/III/nitrate solution.

1,0 ml of 0,1 n $AgNO_3$ equals to 5,845 mg NaCl.

B.4.1.3.7. Free iron/II/ content.

Requirement: at least 8,0 µg/ml

Determination/summarized/.

Reagents:

1. 0,1404 g $Fe/NH_4/_2 \times 6\ H_2O$ + 5,0 ml cc $H2SO_4$ ad 1000 ml distilled water
2. 5x dilution of solution 1.
3. 2 m CH_3COONa solution
4. 0,1% dipiridil in n HCl solution
5. Ascorbic acid

To 10,0 ml of FMM medium 5,0 ml of 3. reagent. 1,0 ml of 4. reagent and 0,1 g of 5. reagent have to be added. After 1 hour standing on a light place, the extinction has to be measured by spectrophotometer at a wavelenght of 480 nm against a control solution containing 1,0 ml

FMM medium and 15,0 ml distilled water.
According the extinction value, the Fe/II/ ion concentration could be read from a calibration curve prepared previously, for its preparation the reagents 1. and 2. are required.

B.4.2. Control of single harvests.

"In process" tests for tetanus toxin.

B.4.2.1. Control for bacterial purity by using Gram stained smears.

B.4.2.2. Determination of Lf content by flocculation method.
For the determination of the Lf value of an unknown sample of tetanus toxin or toxoid, increasing volumes of the reference antitoxin are measured into a series of flocculation tubes and the volume is made up to 1,0 ml with normal saline. To each tube 1,0 ml of the toxin or toxoid under test will be added and properly mixing by shaking. The tubes are then put in a water bath held at 45 C. The tubes are observed continuously and the tube which shows flocculation at first is noted. The Lf value of the toxin or toxoid is calculated from the concentration of toxin and antitoxin contained in this tube.

$$Lf/ml = \frac{A \times B \times \text{dilution rate of toxin or toxoid under test}}{C}$$

A= concentration of reference antitoxin /I.U./ml/
B= quantity of reference antitoxin in ml
C= quantity of toxin or toxoid under test

Note: A,B,C quantities concern the tube showing flocculation at first!

B.4.2.3. Determination of L+ value /limes lethalis/.
The test should be carried out on at least 5 mice weighing 14-16 g at a level of L+/10 which means that one dose of 0,4 ml given to one mouse contains 0,1 I.U. of tetanus antitoxin.
The reference tetanus antitoxin should be diluted to contain 1,0 I.U./ml. From the toxin under test, 1/10 and 1/60 dilutions should be prepared. Incubation time is 1 hour at a dark place at room temperature. After the incubation period, one or more mice have to be injected subcutaneously from each tube. The mice are observed for four days and the

mixture killing the mice on or about the fourth day contains one L+/10 dose of toxin.

Table of the titration

			dil. of the toxin	
saline	tetanus antitoxin 1,0 I.U./ml	10x	60x	L+/ml
2,0	1,0	1,0	-	10
2,5	1,0	0,5	-	20
1,0	1,0	-	2,0	30
1,5	1,0	-	1,5	40
1,8	1,0	-	1,2	50
2,0	1,0	-	1,0	60
2,14	1,0	-	0,86	70
2,25	1,0	-	0,75	80
2,33	1,0	-	0,67	90
2,4	1,0	-	0,60	100

Calculation:

$$L+/ml = \frac{B \times C \times \text{dilution of toxin under test}}{A}$$

A= the volum of toxin in ml/tube
B= quantity of the reference antitoxin in ml
C= concentration of the reference antitoxin in I.U./ml used in the test /1,0/

For further processing of the toxin, a minimum value of 20 L+/ml should be required.

B.4.2.4. Determination of the Minimum Lethal Dose /M.L.D./

The M.L.D.value of the tetanus toxin is that amount of toxin which when injected into mice of about 18-20 g in weight, kills the majority in four days.
For determination, dilutions of the toxin /e.g. 1/10-1/1.000000/ are to be prepared and injected into mice.

B.4.3. Control of bulk purified toxoid.

"In process" tests.

B.4.3.1. Sterility test using thioglycolate and Sabouraud media according to WHO requirements.

B.4.3.2. Specific toxicity test.
Each bulk purified toxoid shall be tested for the presence of tetanus toxin by the injection of at least five guinea-pigs, each weighing between 250 and 300 g. Each guinea-pig shall be injected subcutaneously with 1,0 ml of a dilution of toxoid containing at least 500 Lf of toxoid.
Animals that die shall be examined by autopsy. The bulk purified toxoid shall pass the test if no guinea-pig shows symptoms of specific paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of animals survive the test period.

B.4.3.3. Test for irreversibility of toxoid.
A sample of purified toxoid is diluted in saline containing 0,01% thiomersal to give 300 ml of 10 Lf/ml toxoid.
Aliquots of 100 ml of the diluted toxoid are held at 4^{o}, 20^{o}, and 32^{o} for six weeks. 5 ml of each sample is injected subcutaneously into each of five guinea-pigs weighing between 250 and 300 g. The guinea-pigs are weighed on the first, third and seventh day following injection, then weekly for a further three weeks. The animals should show no signs of tetanus intoxication.

B.4.3.4. Total Combining Power /TCP/ test.
Dilutions of the tetanus toxoid under test, by using Jensen buffer solution, shall be prepared. From each dilution 0,5 ml shall be measured in a dark coloured bottle and 1,0 ml of tetanus antitoxin /diluted to 1,0 I.U./ml/ should be added. This mixture, after shaking well, should be incubated for 24 hours at room temperature. After incubation, to each bottle 10 L+/20 I.U. tetanus toxoid has to be added and the total volume shall be made up to 4,0 ml by using Jensen buffer

solution. For control, into one bottle 0,5 ml of tetanus antitoxin /1,0 I.U./ml/ and 10L+/20 I.U. tetanus toxin shall be measured and made up to a volume of 4,0 ml by Jensen buffer. All bottles /test and control/ should be shaken well and incubated for half an hour at room temperature. Thereafter, from all mixtures 0,4 ml should be injected subcutaneously into mice wighing 16-18 g. /Number of mice 2-4 pro dilutions/.
Observation period: 6 days controlling symptoms of tetanus and deaths.
The Total Combining Power expressed in Binding Units /B.U./ will be determined by the dilution factor of the toxoid under test which causes the death of the mice on the fourth day similarly to that of control animals.

For further processing of the toxoid, a minimum value of 20 B.U./ml should be required.

B.4.3.5. Lf content: see Section B.4.2.2.

B.4.3.6. Test for antigenic purity.
Each bulk purified toxoid shall be tested for antigenic purity by determining the Lf value and the concentration of protein /nondialysable/ nitrogen.
Determination of Protein Nitrogen /PN/ content.
Total Nitrogen /TN/ content could be determinated by Kjeldahl method. It must be calculated for mg/ml value.
Rest Nitrogen /RN/ content could be determined by Kjeldahl method from the supernatant after precipitation by trichloroacetic acid.
The value must be calculated for mg/ml.

$$PN\ mg/ml = TN-RN$$

Minimum purity requirement for tetanus toxoid: 1000 lf/mg PN.

B.4.3.7. Test for pH.

Required value: $7,4 \pm 0,1$

B.4.3.8. Test for residual free formaldehyde.

Requirement: maximum 0,02 %

Determination /summarized/.

The test could be performed by using 0,45% dimedon solution of which 10,0 ml should be given to 5,0 ml toxoid filtrate. After 12 hours incubation period, the mixture has to be filtered and the precipitate shall be dried at 110°C for one day. After treating with acetone, the precipitate shall be weighed and the calculation could be done by using the following formula:

$$\text{Formaldehyde content\%} = \frac{\text{the weight of dried prec./g/} \times Ms_1 \times 100}{Ms_2}$$

$Ms_1 = 30,03$

$Ms_2 = 292,36$

There is also a colorimetric determination of free formaldehyde by using fuchsin-sulfurous acid /WHO Manuel for Tetanus Toxoid, Appendix T 18/.

B.4.4. Control of final bulk.

B.4.4.1. Sterility test according to WHO requirements.

B.4.4.2. Specific toxicity test.
Each final bulk shall be tested for the presence of tetanus toxin by injection into at least 5 guinea-pigs, each weighing 250-300 g. Each guinea-pig shall be given a subcutaneous injection with a quantity equivalent to at least 5 single human doses. Animals that die shall be examined by autopsy. The final bulk shall pass the test if no guinea-pig shows paralysis or any other signs of tetanus within 21 days of injection and if at least 80% of the animals survive the test period.

B.4.4.3. Test for adjuvant content.
Determination of $AlPO_4$ content: Section B.2.1.

B.4.4.4. Test for preservative /thiomersal/ content.
Determination by microbiological means: serial dilutions of a standard thiomersal solution /0,01%/ shall be compared with those prepared in the same way from the toxoid under test by using sufficient Staphylococcus aureus organisms as "indicator". After 24 hours incubation period, the inhibition of bacterial growth has to be compared in the reference and test tubes. The effective concentration is expressed in terms of the dilution of reference thiomersal solution to which the test vaccine mostly closely approximates.
Determination by chemical means: the thiomersal content can be determined spectrophotometrically using diphenylthiocarbazone /dithizone/ reagent. This method is described in WHO Manuel for Tetanus Toxoid, Appendix T.16.

B.4.4.5. Test for residual free formaldahyde: Section B.4.3.8.

B.4.4.6. Test for pH.
Requirement between 6,0-7,0.

B.4.4.7. Potency test.
Each final bulk shall be tested for immunizing potency by comparison with a national reference material calibrated

against the appropriate international standard. The test involves the inoculation of groups of guinea-pigs /weighing 250-350 g/ or mice /wighing 14-20 g, provided that, in a single test, the individual weights of the mice shall not vary by more than 3 g/. Three to four dilutions of both the final bulk and reference material shall be used. After immunization /21-28 days/, the animals shall be challenged with a lethal /50-200 LD_{50}/ challenge dose of tetanus toxin given subcutaneously. Standard statistical methods /Reed-Muench, Spearman-Kärber, Probit analysis/ shall be applied to calculate the potency of the final bulk by comparing the ED_{50} values. Sufficient animals should be used to achieve a 95% confidence interval smaller than 50-200%.
Minimum requirement: 40 I.U./ single human dose.

B.4.4.8. Stability test.

The stability of the vaccine should be proved by testing at least three consecutive batches of final bulk during storage. The vaccine should meet requirements for potency up to the expiry date, provided that it has been stored at the recommended temperature. Accelerated stability tests by performing heat degradation could be used respectively.

B.4.4.9. Test for adsorption.

The detection of non-adsorbed toxoid could be done by centrifugation of a sample of vaccine and determination of the quantity of toxoid in the supernatant could be carried out either by flocculation or TCP tests /B.4.2.2., B.4.3.4./.
The quantity of non-adsorbed toxoid should be less than 20% of the total toxoid added to the adjuvant.

B.4.5. Control tests on final lot.

B.4.5.1. Sterility test.
Each final lot shall be tested for sterility according to WHO requirements.

B.4.5.2. Potency test: according to Section B.4.4.7. if such a test has not been performed on the final bulk.

B.4.5.3. Innocuity test.
Each final lot shall be tested for abnormal toxocity by the injection of one human dose, but not more than 1,0 ml into each of 5 mice /weighing 17-22 g/ and at least one human dose, but not more than 1,0 ml into each of 2 guinea-pigs /wighing 250-350 g/ by the intraperitoneal route. /In some countries, subcutaneous injections are administered/.
The final product shall be considered as innocuous if the animals survive for at least 7 days without showing any signs of toxicity.

B.4.5.4. Test for pH.
Requirement between 6,0-7,0

B.4.5.5. Test for adjuvant /$AlPO_4$/ content: Section B.2.1.

B.4.5.6. Test for preservative /thiomersal/ content: Section B.4.4.4. /In some countries, this test is applied either on the final bulk or on the final lot only/.

B.4.5.7. Inspection of final containers.
Each container in each final lot shall be inspected visually, and those showing abnormalities, such as e.g. clumping or the presence of particles of any kind, shall be discarded.

B.4.5.8. Identity test.
An identity test shall be performed on at least one labelled container from each final lot. Flocculation of the toxoid with tetanus antitoxin may serve as an identity test. Tests on toxoid containing an aluminium carrier could be carried out

after the carrier has been dissolved with a solution of sodium citrate or after the toxoid has been eluted by a suitable method. If the carrier can not be removed, tests may be made by specific antitoxin neutralization or by antitoxin production in animals.

B.5. Diphtheria-Tetanus Vaccine Adsorbed.

Because of the production of this vaccine will be formulated in LANAVET from imported diphtheria antigen, quality requirements for this antigen will be given here beginning at the bulk purified toxoid.

B.5.1. Diphtheria bulk purified toxoid.

"In process" tests.

B.5.1.1. Sterility test: according WHO requirements.

B.5.1.2. Specific toxicity: Section B.4.3.2.
The material shall pass the test if no guinea-pig shows symptoms of specific intoxication within 6 weeks of injection and if at least 80% of the animals survive the test period.

B.5.1.3. Test for irreversibility of toxoid: Section B.4.3.3.

B.5.1.4. Lf content: Section B.4.2.2. /by using diphtheria antitoxin/.

B.5.1.5. Test for antigenic purity: Section B.4.3.6.
Minimum requirement: 1500 Lf/mg PN.

B.5.1.6. Test for pH.
Required value: $7,4 \pm 0,1$

B.5.1.7. Test for residual free formaldehyde: Section B.4.3.8.

Note: detailed production and control protocol of the imported diphtheria antigen will be strictly required from the supplier! Based on this protocol, not all the above tests should be performed in LANAVET /if accepted by the National Control Authority/.

For the tetanus antigen, produced in LANAVET for the Diphtheria-Tetanus vaccine, requirements and methods listed in Sections B.4.1., B.4.2. and B.4.3. shall be applied.

B.5.2. Control of final bulk.

B.5.2.1. Sterility test: according to WHO requirements.

B.5.2.2. Specific toxicity: Section B.4.4.2.
Requirement: no any symptoms of specific intoxication within 6 weeks of injection.

B.5.2.3. Test for adjuvant /$AlPO_4$/ content: Section B.2.1.

B.5.2.4. Test for preservative /thiomersal/ content: Section B.4.4.4.

B.5.2.5. Test for residual free formaldehyde: Section B.4.3.8.

B.5.2.6. Test for pH
Required value: between 6,0-7,0

B.5.27. Potency test: Section B.4.4.7. /By using only groups of guinea-pigs for the diphtheria component/.

B.5.2.8. Stability test: Section B.4.4.8.

B.5.2.9. Test for adsorption: Section B.4.4.9.
Both diphtheria and tetanus antigens should be determined by means of flocculation method.

B.5.3. Control tests on final lot.

B.5.3.1. Sterility test: according to WHO requirements.

B.5.3.2. Potency test: B.5.2.7. /if not done on final bulk/.

B.5.3.3. Innocuity test: Section B.4.5.3.

B.5.3.4. Test for pH. Requirement: between 6,0-7,0

B.5.3.5. Test for adjuvant /$AlPO_4$/ content: Section B.2.1.

B.5.3.6. Test for preservative /thiomersal/ content: Section B.4.4.4.

B.5.3.7. Inspection of final containers: Section B.4.5.7.

B.5.3.8. Identity test: Section B.4.5.8. /Both for diphtheria and tetanus antigens/.

B.6. Diphtheria-Pertussis-Tetanus Vaccine Adsorbed.

The production of this vaccine, concerning the diphtheria and pertussis components, will be formulated in LANAVET from imported antigens. The quality requirements for the pertussis antigen will be given here beginning at the bulk concentrated suspension. Regarding the tetanus and diphtheria antigens, requirements and methods listed in Sections B.4.1., B.4.2., B.4.3. and B.5.1. shall be applied.

B.6.1. Pertussis bulk concentrated suspension.

"In process" tests.

B.6.1.1. Sterility test: according to WHO requirements.

B.6.1.2. Specific toxicity /mouse weight gain test/.
The pertussis bulk concentrated suspension shall be diluted with saline to contain 15 I.O.U. /equals to one single human dose/. Male and female mice,weighing 14-16 g, are weighed in groups of not less than 10 and injected intraperitoneally with half of a single human dose in a volume of 0,5 ml.
A control group of mice is injected with an equal volume of 0,9% saline. Both groups are weighed on the first, fourth and seventh day after injection. A pertussis suspension is considered non toxic if, at the end of 72 hours the group wight is not less than that at the time of injection, and at the end of seven days the average weight gain per mouse is not less than 60% of that of the control group of mice, in addition no vaccine related deaths should occur. If any mice die the test shall be repeated and the aggregate deaths may not exceed 5%.

B.6.1.3. Potency test.
The potency test of the bulk concentrated suspension shall be determined by a comparative assay in relation to that a reference material calibrated against the Intrnational Standard for Pertussis Vaccine. The assay shall be performed by immunizing groups of mice /weighing 14-16 g/ intraperitoneally and by intracerebral challenge after a period of 14-17 days.

Standard statistical methods /Reed-Muench, Wilson-Worcester, Probit analysis/ shall be used to calculate the potency of the bulk suspension by comparing ED_{50} values. Sufficient animals should be used to achieve a 95% confidence interval smaller than 64-156%.
Minimum requirement: 8 I.U./30 I.O.U.

B.6.1.4. Test for opacity, by comparing with the International Opacity Reference Preparation.

B.6.1.5. Test for pH.
Required value between 6,6-6,8.

Note: detailed production and control protocol of the imported pertussis antigen will be strictly required from the supplier!
Based on this protocol, not all the above tests should be performed in LANAVET /if accepted by the National Control Authority/.

B.6.2. Control of final bulk.

B.6.2.1. Sterility test: according to WHO requirements.

B.6.2.2. Specific toxicity.
For diphtheria and tetanus components: Section B.5.2.2.
For pertussis component: Section B.6.1.2.

B.6.2.3. Test for adjuvant /$AlPO_4$/ content: Section B.2.1.

B.6.2.4. Test for residual free formaldehyde: Section B.4.3.8.

B.6.2.5. Test for preservative /thiomersal/ content: Section B.4.4.4.

B.6.2.6. Test for pH.
Required value: between 6,0-7,0

B.6.2.7. Potency test.
For diphtheria: Section B.5.2.7.
For pertussis: Section B.6.1.3.
For tetanus: Section B.4.4.7.

B.6.2.8. Stability test: Section B.4.4.8.

B.6.2.9. Test for adsorption: Section B.5.2.9. /Centrifugation of pertussis bacteria should be needed/.

B.6.3. Control tests on final lot.

B.6.3.1. Sterility test: according to WHO requirements.

B.6.3.2. Potency test: Sections B.5.2.7., B.6.1.3. and B.4.4.7.
/If not done on final bulk/.

B.6.3.3. Innocuity test: Section B.4.5.3.

B.6.3.4. Test for pH
Required value: between 6,0-7,0

B.6.3.5. Test for adjuvant /$AlPO_4$/ content: Section B.2.1.

B.6.3.6. Test for preservative /thiomersal/ content: Section B.4.4.4.

B.6.3.7. Inspection of final containers: Section B.4.5.4.

B.6.3.8. Identity test.

For diphtheria and tetanus antigens: Section B.4.5.8.

For pertussis antigen, agglutination of the bacteria with specific antipertussis serum should be used. In some countries, Gram stained smear of the vaccine is used for the pertussis identity.

Summarized quality requirements for Tetanus Toxoid, Diphtheria-Tetanus and Diphtheria-Pertussis-Tetanus Adsorbed Vaccines.

Tetanus toxoid vaccine adsorbed.

Sterility	sterile
Potency	at least 80 I.U./ml
Innocuity	innocuous
pH	6,0-7,0
Aluminium	1,3-1,5 mg/ml
Thiomersal	0,01%
Formaldehyde	0,02% /maximum value/
Physical properties	free from clumping or other particles
Identity	identical

Diphtheria-tetanus vaccine adsorbed.

Sterility	sterile
Potency	diphtheria at least 60 I.U./ml
	tetanus at least 80 I.U./ml
Innocuity	innocuous
pH	6,0-7,0
Aluminium	1,3-1,5 mg/ml
Thiomersal	0,01%
Formaldehyde	0,02% /maximum value/
Physical properties	free from clumping or other particles
Identity	diphtheria: identical
	tetanus: identical

Diphtheria-pertussis-tetanus vaccine adsorbed.

Sterility	sterile
Potency	diphtheria at least 60 I.U./ml
	pertussis at least 8 I.U./ml
	tetanus at least 80 I.U./ml /on guinea-pigs/
	at least 120 I.U./ml /on mice/
Innocuity	innocuous
pH	6,0-7,0

Aluminium	1,3-1,5 mg/ml
Thiomersal	0,01%
Formaldehyde	0,02% /maximum value/
Physical properties	free from clumping or other particles
Identity	diphtheria: identical
	pertussis: identical
	tetanus: identical

C. Local implementation of quality control requirements in LANAVET.

C.1. General.

The quality control facility for vaccines must be independent of the production area. This is true not only for the building/s/, but also for the staff. This structure gives an independent opinion of the safety and efficacy of each batch of vaccine and the reports of such a control laboratory must be presented directly to the Director of the whole vaccine production and control facility or to the National Control Authority /if existent/.
This does not necessarily mean that the building has to be a separate one. Areas within a single building for both production and quality control are acceptable, provided that they are adequately isolated. Such a construction may reduce building costs and make the supply of services more efficient.
It is important that the quality of the building, laboratories, equipment and staff are at least as good as those of production area: the quality control facilities must be capable of carrying out all tests applicable to the vaccines. In addition, they should be equipped to effect the quality control of the starting materials, such as media, control of quality of water, determination of the concentration of additives, such as adjuvants, preservatives etc. Therefore, a laboratory for chemical tests, beside the biological one, is absolutely necessary.
In LANAVET, where the production of bacterial and viral vaccines for veterinary use is performed on a very high level, the control facilities of these vaccines are providing a suitable base for the implementation of the quality control tests and methods applicable to human vaccines. /TT, DT, DPT/.
The quality control of veterinary vaccines are carried out in the facilities of the Departments Immunology-Quality Control, Biochemistry and in the animal houses.
In the following paragraphs, characterization of the present situation and recommandations for some modifications will be given aiming the succesful implementation of the quality control of human vaccines.

C.2. The existing control facilities.

In general, the laboratories and animal housing rooms for the control of the veterinary vaccines are well isolated from those of vaccine production and their equipment and staff is suitable for performing the necessary tests and methods required for the control of veterinary vaccines.

C.2.1. Immunology-Quality Control Department.

C.2.1.1. Rooms and equipments.

Laboratory 20 m^2, equipped by

1 pc. centrifuge /Jouan/
2 pcs.Water-bath
1 pc. light microscope /Olympus/
1 pc. refrigerator
1 pc. balance /electronic, Sartorius/
2 pcs.magnetic stirrer
1 pc. pH meter
1 pc. box for sterility testing

Laboratory, 13 m^2, for the injection of small laboratory animals.

Walk-in incubator, 37^oC
Walk-in cold room, 4^oC
} used by other departments, too.

Office, 10 m^2, for the chief of the section.

Facilities and equipments of animal houses, which belong to this department, will be mentioned in paragraph C.2.3.

C.2.1.2. Activity of the department.

Tests for the sterility, innocuity, and potency of veterinary vaccines.

Diagnostics of animal diseases using serological technics /ELISA, agglutination, haemagglutination-inhibition, complement fixation etc./

C.2.1.3. Staff.

1 veterinary doctor
1 senior technician
3 laboratory aids

C.2.2. Biochemistry Department.

C.2.2.1. Rooms and equipments.

Total size of the department is about 60 m^2 devided by glass walls into four rooms, each about 15 m^2.

Office for the chief of the section.

Equipment of three laboratory rooms:

- 1 pc. spectrophotometer /Spectronic 2000/
- 1 pc. apparatus for gas-chromatography /Enica 21/
- 1 pc. apparatus Büchi /for determination of N content/
- 1 pc. equipment for electrophorises /Celloprofil/
- 1 pc. apparatus for measuring residual moisture by using Karl-Fischer method
- 1 pc. flame-photometer /Corning 410 C/
- 1 pc. evaporator rotative
- 1 pc. water distiller
- 1 pc. centrifuge /Jouan/
- 1 pc. refrigerator
- 1 pc. light microscope /Olympus/
- 1 pc. balance of precision
- 1 pc. balance Roberval
- 3 pcs.pH meter

C.2.2.2. Activity of the department.

Service for clinical diagnostic

Service for analyzing foods of animal origin and foods destined for animal feeding

Physical and chemical control of veterinary vaccines produced by LANAVET /determination of adjuvant, formaldehyde and moisture/.

C.2.2.3. Staff.

- 1 veterinary doctor
- 2 senior technicians
- 2 technicians
- 2 laboratory aids

C.2.3. Animal housing.

In LANAVET, being an institute for the production and control of veterinary vaccines, there is a big area for animal housing, well separated from other facilities. The "big" animals /beefs, horses, goats, sheeps, swines/ are housed in isolated premises. Because of in the production and quality control of human vaccines only "small" laboratory animals are used, this paragraph is dealing with their facilities.

C.2.3.1. The house for guinea-pigs.

This house, consisting of one room only, has a surface of 64 m^2. /Annex 1./. The guinea-pigs are kept and breeded on the floor on a territory of about 25 m^2, in the left side of the room. They are feeded by pellets, vegetables and water. On the right side, there are cages with rats, used by the Virology Department for experiments. The room is used also for the storage of food and bedding /pellets, hay and vegetables/.
The house has one entrance, four windows and supplied with tap water, electricity and air-conditioning equipment.

C.2.3.2. The house for mice.

This house consists of one big room of about 120 m^2 and a storage room of 12 m^2. /Annex 2. /. The mice are kept and breeded in plastic cages, feeded by pellets and water. There is a number of rabbits held in single cages in the room as well. The house has one entrance, six windows and supplied with tap water, electricity and air-conditioning equipment.
The house is surrounded by a deep ditch to defend the mice from snakes.

C.2.3.3. There is a separate house consisting of two rooms which are isolated by wall. Each surface is 20 m^2. /Annex 3./. In one room some chicken are held, the other is the room of the supervisor. Both rooms have separated entrances from the yard and are supplied with tap water and electricity.

C.2.3.4. The house for "egg-laying".
Big room of about 200 m^2. /Annex 4./. The room is quite empty, it is used, at present, for nothing.
The house has two entrances, twelve windows and is supplied with electricity and six/!/ air-conditioning equipment.

C.2.3.5. Belonging to the Immunology Department, there is a well separated room for autopsy /30 m^2/ and two incinerators.

C.3. Recommended modifications and suggestions.

As it was already defined in previous paragraphs, the quality control of the human vaccine /TT, DT, DPT/ should be performed within the existing facilities of LANAVET, which are suitable, in general, for the introduction of new control methods according to WHO requirements. In spite of this fact, some modifications regarding organization, rooms, equipment and staff, are recommended.
A well organized quality control system must be regarded as one of the most important basis of any human vaccine production. In LANAVET, the quality control of veterinary vaccines is carried out in two independent departments, called "Immunology" and "Biochemistry". According to WHO recommendations, the establishment of an independent Quality Control Division, consisting of biological and chemical control units, including its own animal housing premises, is highly recommended. This reorganization does not mean fundamental changes of the present situation. The rooms, equipments and staff of the existing departments should be concentrated in the new Division and the biological and chemical units could be headed by their present deputy chiefs. The departments "Immunology" and "Biochemistry" could be called in the future as follows:

Quality Control /Biology/

Quality Control /Biochemistry/

Final release of the human vaccines will be done by the chief of the biological control section, taking in account the chemical results as well. In the farther future, when the routine production of TT and the formulation /or production/ of DT and DPT vaccines will be

completed, the supervising of all quality control activities, including the biological and chemical ones, by a general chief of the Quality Control Division, should be considered.

In the frames of the existing departments, the following modifications are recommended.

C.3.1. Quality Control /Biology/ at present "Immunology".

C.3.1.1. Rooms and equipments.

The existing laboratory with its equipment is suitable to perform the biological control tests of human vaccines, excepting the sterility testing. The box which is used for the sterility testing of veterinary vaccines, is not suitable for the human ones. For this purpose, an isolated sterile room has to be installed. A solution could be found, when the laboratory, presently used for the injection of laboratory animals, could be modified for sterility testing.
A recommended layout for the remodelling of this room is presented on the next page.

In the sterile room, the following equipments have to be purchased and installed:

1 pc. LAF cabinet
2 pcs. Laboratory thermostat /22-24^{o}C and 30-32^{o}C/.

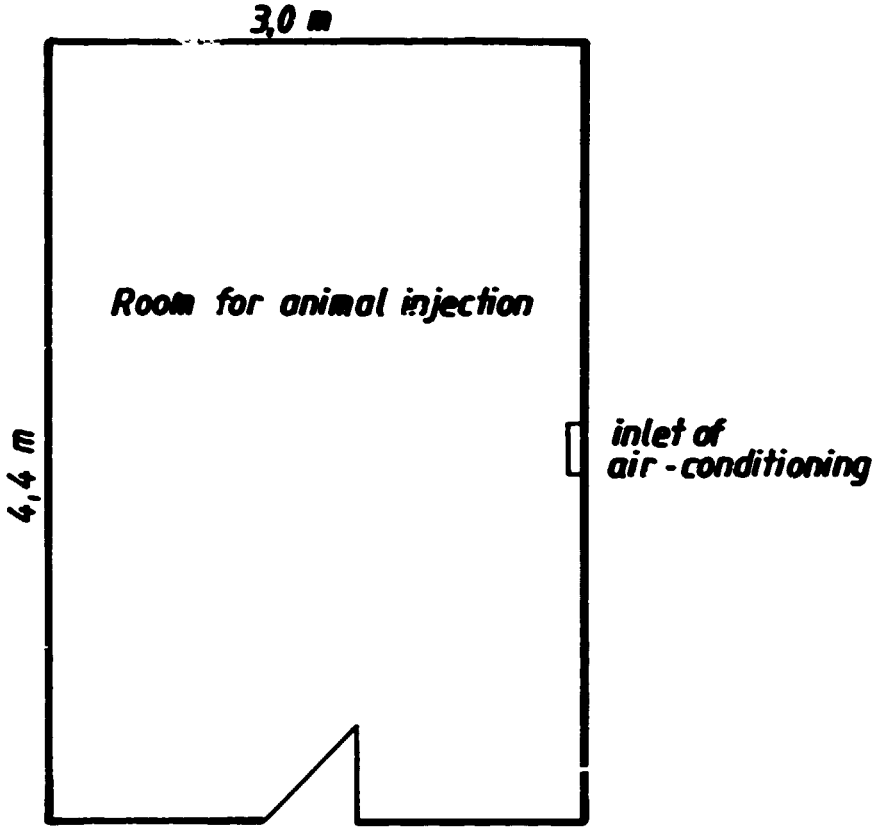
The inlet of air conditioning shall remain in the sterile room, on its present place.

For the injection of animals /if necessary/, an other room is available. General laboratory instruments and materials will be requested.

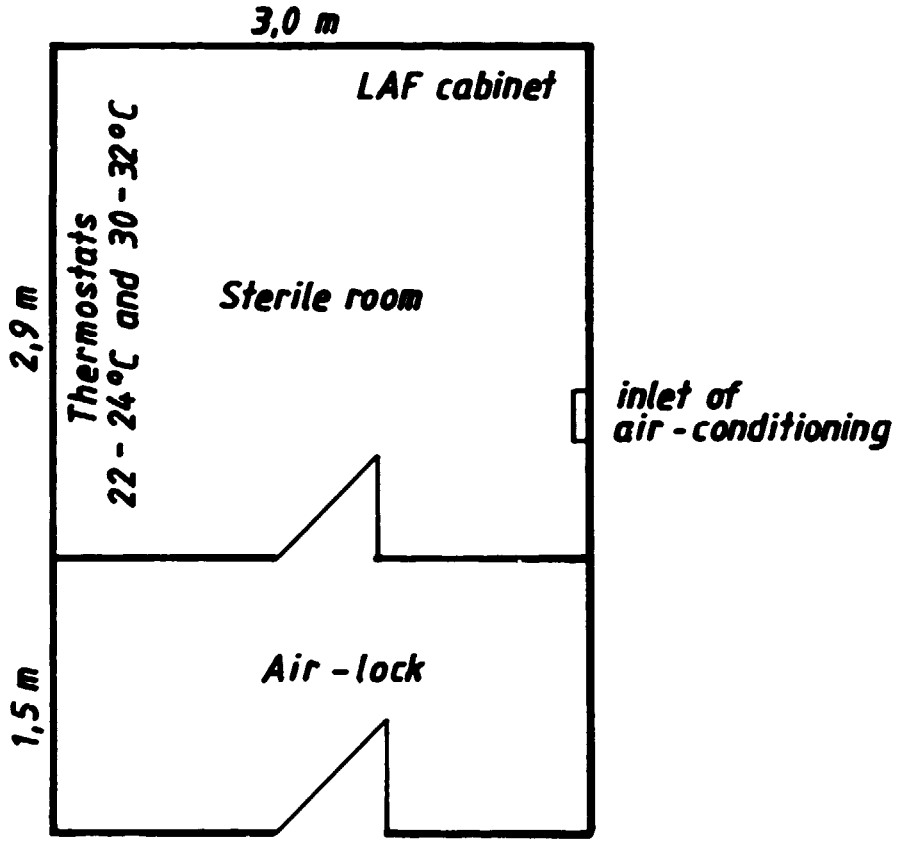
C.3.1.2. Activity.

After the introduction of the production of human vaccines, the control activity of the department shall be extended significantly. All biological control tests of human vaccines must be performed according to WHO requirements.

LAYOUT AT PRESENT



LAYOUT AFTER REMODELLING



Scale 1:40

Summarized list of tests to be introduced /detailed description in chapter "B"./

Tetanus Toxoid Vaccine.

B.4.3.1. Sterility test /including microbiological control of media as well/.
B.4.3.2. Specific toxicity test
B.4.3.3. Test for irreversibility of toxoid
B.4.3.4. Total Combining Power test
B.4.3.5. Lf content
B.4.4.1. Sterility test
B.4.4.2. Specific toxicity test
B.4.4.4. Test for preservative content
B.4.4.7. Potency test
B.4.4.8. Stability test
B.4.4.9. Test for adsorption
B.4.5.1. Sterility test
B.4.5.3. Innocuity test
B.4.5.7. Inspection of final containers
B.4.5.8. Identity test

Diphtheria-Tetanus Vaccine.

B.5.1.1. Sterility test
B.5.1.2. Specific toxicity test
B.5.1.3. Test for irreversibility of toxoid
B.5.1.4. Lf content
B.5.2.1. Sterility test
B.5.2.2. Specific toxicity test
B.5.2.4. Test for preservative content
B.5.2.7. Potency test
B.5.2.8. Stability test
B.5.2.9. Test for adsorption
B.5.3.1. Sterility test
B.5.3.3. Innocuity test
B.5.3.7. Inspection of final containers
B.5.3.8. Identity test

Diphtheria-Pertussis-Tetanus Vaccine

B.6.1.1. Sterility test
B.6.1.2. Specific toxicity test
B.6.1.3. Potency test
B.6.1.4. Test for opacity
B.6.2.1. Sterility test
B.6.2.2. Specific toxicity test
B.6.2.5. Test for preservative content
B.6.2.7. Potency test
B.6.2.8. Stability test
B.6.2.9. Test for adsorption
B.6.3.1. Sterility test
B.6.3.3. Innocuity test
B.6.3.7. Inspection of final containers

In addition to the above activities, the control of the packaging materials /labels, signatures, leaflets/ will also be required.

C.3.1.3. Staff.

To perform all the above WHO requirements, the strengthening of the biological control department is strongly recommended by recruiting of new staff members:

1 medical doctor or pharmacist
1 senior technician
2 laboratory aids

This strengthening should be achieved before the completion of the human vaccine production.

The oversea training of the present personnel in the facilities of the Institute providing technology is recommended as follows:

Deputy head of "Immunology"	3 months
Senior technician of "Immunology"	3 months

Note: see also chapter XI./in the Report of 15.11.1988/

After finishing the training programmes abroad, a follow-up local training in LANAVET is highly recommended including all quality control staff members.

C.3.2. Quality Control /Biochemistry/ at present "Biochemistry".

C.3.2.1. Rooms and equipments.

The existing laboratories and equipment are sufficient to introduce chemical control of human vaccines. The purchase of some reagents and chemicals is requisted to perform the new tests.

C.3.2.2. Activity.

After the introduction of the production of human vaccines, the activity of the present department will be extended. All chemical control tests of human vaccines must be performed according to WHO requirements.

Summarized list of tests to be applied /detailed description in chapter "B"/.

B.2. Control of starting raw materials
B.2.1. Determination of Aluminium-phosphate gel
B.2.2. Chemical control of distilled water
B.3. Chemical control of filling materials /vials, rubber-stoppers/

Tetanus Toxoid Vaccine.

B.4.1.3.4. Alpha-amino N content
B.4.1.3.5. Glucose content
B.4.1.3.6. NaCl content
B.4.1.3.7. Free iron/II/ content
B.4.3.6. Test for antigenic purity
B.4.3.8. Test for residual free formaldehyde
B.4.4.3. Test for adjuvant content
B.4.4.6. Test for pH

Diphtheria-Tetanus Vaccine.

B.5.1.5. Test for antigenic purity
B.5.1.7. Test for residual free formaldehyde

B.5.2.3. Test for adjuvant content
B.5.2.6. Test for pH

Diphtheria-Pertussis-Tetanus Vaccine.

B.6.2.3. Test for adjuvant content
B.6.2.4. Test for residual free formaldehyde
B.6.2.6. Test for pH

C.3.2.3. Staff.

To perform all the above WHO requirements, the present staff seems to be sufficient.
Because of different methods of chemical control should be used for human vaccines, as some of those performed at present by the department, an oversea training in the facilities of the Institute providing technology is recommended as follows:

Deputy head of "Biochemistry" 2 months

Note: for the time scheduling of all biological and chemical quality control activities, see chapter XII.
/in the Report of 15.11.1988/

C.3.3. Animal housing.

For a number of quality control tests of human vaccines, healthy mice and guinea-pigs are requested for potency and safety tests according to WHO requirements. A well organized animal house is the basis of many biological control activities. In LANAVET, the housing of mice and guinea-pigs, compared to the very up-to-date organization and equipment of the vaccine production and veterinary diagnostic areas, seems not to be optimal. With some modifications of the existing buildings, better conditions could be achieved on this very important area.

As a follow-up of reorganization, one building should be joined to the facilities of the biological control department, including its staff and equipment, utilized exclusively by quality control authorized personnel. For this purpose, taking in account other alternatives as well, the remodelling of the existing house for mice is recommended.

C.3.3.1. Remodelling the house of mice.

In this house, the holding of all test animals /mice and guinea-pigs/ should be performed. The big room could be devided by a corridor, separating mice and guinea-pigs, to minimize the risk of any cross contamination among the different kind of animals. Isolated by walls of suitable materials, the creation of seven rooms is recommended.

Room for mouse potency tests
Room for mouse safety tests
Room for mouse holding /and breeding?/
Room for guinea-pig tests
Room for guinea-pig quarantine, as they will be transported from Ngoundéré or from somewhere else
Room for cage disinfection and washing
Room for storage /existing, for food and bedding/

The size of each room is about 12,5 m^2 but that for guinea-pig tests is about 25 m^2. All rooms should be installed with airconditioning facility to promote environmental stability. In all animal rooms, the existing windows should remain on their present places providing natural light, but arteficial lighting for all rooms is also required.
A suitable quantity of racks
cages /of different sizes/
movable working tables
containers /to be closed/
lids with feeders
water bottles with rubber stoppers and sipper tubes
should be purchased.

A recommended layout for the remodelling of this room is presented on the next page.

C.3.3.2. The house of guinea-pigs.

In this house the biological quality control tests of veterinary vaccines could be concentrated /mice, guinea-pigs, rats/. For this reason, a remodelling seems to be necessary, its layout should be prepared by LANAVET specialists.
The establishment of a room for the breeding of mice is recommended.

C.3.3.3. In the separate house, where the supervision and administration is performed, there is a room /"Eclosoir" in French/ with some chicken, which could be placed elswhere.
This room, after a small remodelling, could be served for the mouse tests of tetanus toxin production /determination of L+ and DLM values/.
As required generally, this test-room should be separated from all others because of possible contamination with Cl. tetani spores. The room should be equipped with

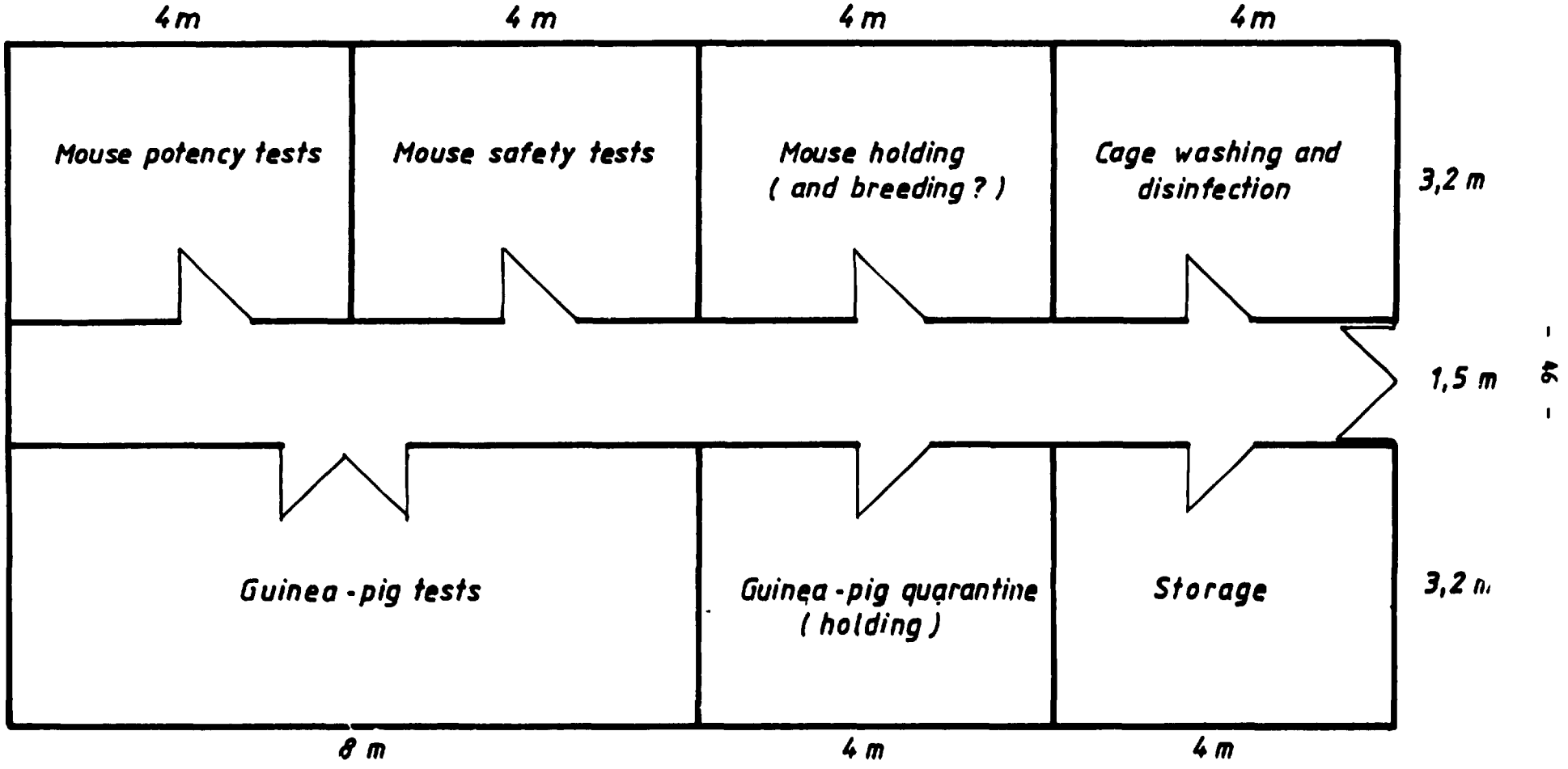
air-conditioning facility and supplied with closed containers for transporting the contaminated animals and materials /bedding/ to the incinerator.
This room must belong to the tetanus toxin producing department, and has to be used only by authorized personnel.

C.3.3.4. The house for "egg-laying".

The big room of about 200 m^2 is used for nothing. As an alternative modification for the farther future, the establishment of a complete breeding-holding-testing facility for all laboratory animals, using clean and dirty corridor system, recommended by the WHO, could be considered.
Regarding the number of laboratory animals required for the quality control of TT, DT, DPT vaccine batches, detailed in chapter III. the remodelling of this house in the near future is not recommended.

Note: for the preparation of the final engeneering layout of the remodelled animal housing facilities, the recommendations of WHO Manuel BLG/UNDP/78.1. should be taken into consideration.

HOUSE OF MICE AND GUINEA-PIGS
(AFTER REMODELLING)



Scale 1:66

(PRESENT LAYOUT : ANNEX 2)

D. The National Control of Vaccines.

D.1. General.

According to WHO requirements, in all countries, where vaccines are administered, the ministry of health is responsible for the suitability of their safety and potency. It is important, therefore, for all such ministries in all countries to establish or recognize pharmacopoeial standards or have access to a means of exercising quality control over biological substancies /i.e. vaccines/. Such a control is generally the responsibility of a legally constituted national control authority which may differ from one country to another.

This authority may be:

the minister of health or other appropriate minister,
a designated national controller; or
the director of a national laboratory concerned with the controls of biological products.

The national control authority should be empowered:

to establish criteria for the acceptability of the products;
to license manufacturers and their products;
to establish standard preparations for the biological assays;
to establish the technical facilities and other mechanisms needed for implementing the requirements.

The facilities that can be established by a national control authority will depend on the technical and financial resources available. It is important, however, to have some facility as national control laboratory, no matter how modest, or to make the necessary arrangements with a comprehensive control system.
The national control laboratory furnishes the necessary technical services to the national control authority. In some cases the director of the laboratory may also be the legally empowered authority.
It is part of the task of the national control authority, in collaboration with the national control laboratory /if existent/

to formulate the requirements to be used in a country and to control the locally produced or imported vaccines for human use. In the developing countries, where all resourses /technical, personel, financial and others/ are quite limited, a step by step approach to the establishment of the national control system is recommended.

As a first stage, a functioning national control authority for biological substances should be established. The simplest and least expensive step would be to appoint a technical adviser to the national control authority. Although such a service would have its limitations, it is worth while in countries with no suitable laboratory facilities.
At this stage, all that is envisaged is that a competent person should be available to act in an advisory capacity.
He may be designated a technical adviser.
His advice will be in the form of recommendations to accept or reject particular batches for use, to demand further information or to require the repetition of tests.
In order to fulfil his functions he should be empowered to:

a./ Establish that a product has come from a suitable source.
b./ Ensure that each batch of the product conforms to the relevant international and national requirements, where these exist.
c./ Evaluate the protocols of manufacture and testing required from the manufacturer. If the product is manufactured in a country where there is only a technical adviser and no quality control facilities, the adviser should arrange through WHO for samples to be tested independently.
The checking of protocols is a most important part of the control of biological products. It r quires meticulous attention to detail and a knowledge of the methods used in the production and control of each of the products under consideration.
d./ Inspect samples of the products, their labelling and accompanying documentation.

e./ Inspect premises and all processes of manufacture including control in the technical adviser's own country and elsewhere when feasible. It can not be overemphasized that inspection by persons knowledgeable in the manufacture of biologicals /i.e. vaccines/ contributes greatly to ensuring the quality of biological products.
For imported products the technical adviser of the importing country should have the right to demand certificates of conformity with any existing national requirements of the country of origin.
To fulfil all these duties, the technical adviser should be a medical or science graduate with qualifications in microbiology and immunology. Its training abroad is highly recommended.

The next step would be to set up laboratory services, though not necessarily with animal facilities since these are very expensive and much can be achieved by in vitro tests.
Every national control laboratory should deal with biological products manufactured in the country itself as well those that are imported. The national control laboratory should be autonomous and administered by, or on behalf of the national control authority, even though, as in some countries, the NCL is housed in a national laboratory that has other functions - i.e. teaching, reasearch, diagnostic microbiology etc.

Quality requirements of final lots of vaccines used in EPI are desccribed in this report. /Diphtheria, Tetanus, Pertussis, BCG, Measles, Poliomyelitis and Rabies vaccines/.
A number of in vitro tests detailed in the report should be performed as:

a./ virus titre of live virus vaccines in cell cultures /control of cold chain facilities!/
b./ determination of viable units in BCG vaccines
c./ identity tests

d./ flocculation tests
e./ determination of protein, residual moisture, adjuvant content, pH etc.

The laboratory should advise the national control authority regarding inspection of manufacturing establishments, or, possibly carry out inspections of such establishments if assigned this responsibility.

The ultimate step is the comprehensive quality control facility that is capable of carrying out all tests on all vaccines, prescribed in WHO requirements.
Some of the control tests are performed on animals. For this reason, the availability of good-quality laboratory animals /mice, guinea-pigs, rabbits/ and of a well equipped animal house are absolutely essential.

In addition to those mentioned above, the functions of a comprehensive laboratory should include performance of the following control tests:
sterility, identity, innocuity, pyrogenicity, safety, potency, stability.

In addition, provision should be made for research and development of new and improved methods in quality control.

The director or officer in charge of the above laboratories should be a medical, pharmaceutical or other science graduate with qualifications in microbiology and immunology.
A broad technical knowledge of the production and control of biological substances /i.e. vaccines/ is indispensable and for this reason a training abroad in Institutions with high reputation is absolutely necessary.

D.2. Control tests required for BCG vaccine

Final product

1. Identity test /e.g. stained smears/
2. Tests for contaminating microorganisms
   /bacterial and mycotic/
3. Safety tests
   a. test for absence of virulent mycobacteria
   b. test for skin reactions in guinea-pigs
4. Test for total bacterial content
   /dry weight of organisms or opacity method/
5. Test for viability
6. Stability test
7. Production consistency

Control tests required for inactivated poliomyelitis vaccine

Final product /trivalent plain vaccine or combined vaccine/

1. Identity test /potency test may be used/
2. Sterility tests /bacterial and mycotic sterility/
3. Innocuity test /in mice and guinea-pigs/
4. Potency test
   a. in vivo /in rats or other suitable animals/
      Neutralization titers to be compared with Reference preparation.
   b. in vitro /D-antigen test by gel diffusion, ELISA, complement fixation, RIA or other suitable tests/
5. Protein nitrogen content. Maximum 10 µg/dose

Control tests required for poliomyelitis vaccine /oral/

1. Identity test /serologically/
2. Test for bacteria and fungi
3. Virus titration /in terms of PFU/ml or $TCID_{50}$/ml/
4. Innocuity tests /in mice, guinea-pigs or rabbits/
5. Stability /37°C, 7 days/

Control tests required for measles vaccine /live/

Final product

1. Identity test /tissue-culture neutralization test or haemagglutination inhibition test using specific antiserum/
2. Sterility test /bacterial and mycotic sterility/
3. Test for virus concentration /on tissue cultures using reference preparation for comparison/ 2 weeks
4. Innocuity test /in mice and guinea-pigs/
5. Protein content /animal-bovine-serum proteins, counter-current electrophoresis/
6. Inspection of final containers
7. Stability /1 week 37°C/ - 3 weeks

Control tests required for measles vaccine /inactivated/

Final product

1. Identity test /potency test may be used/
2. Sterility test /bacterial and mycotic sterility/
3. Potency test /antibody production in groups of guinea-pigs, using reference vaccine/
4. Innocuity test /on mice and guinea-pigs/
5. Protein content /bovine serum/
6. Inspection of final containers

Control test required for cell culture rabies vaccine

Final product /lyophilized/

1. Identity test /potency test may be used/
2. Sterility tests /bacterial and mycotic sterility/
3. Innocuity test /on mice and guinea-pigs/
4. Potency test /NIH test/ 4 weeks
5. Stability test /4 weeks on 37°C, NIH test/ 8 weeks
6. Residual moisture
7. Inspection of final containers
8. Test for pyrogenicity /in rabbits or LAL test/
9. BPL-content /bovine serum protein/
   /counter-current electrophoresis/

D.3. Local possibilities to introduce the national control of human vaccines.

Although human vaccine production and quality control should be introduced in LANAVET /Garoua/, the establishment of the national control facilities is recommended is the capital Yaoundé. Here, some institutions /OCEAC, ONAPHARM, Institut Pasteur/ were inspected to find out the best possobilities for the above purpose.

As the first step, the National Control Authority should be established within the frame of the Ministry of Public Health. As detailed in the paragraph D.1., the minimum requirement is the appointment of a Technical Adviser, whose duties are also detailed in the same paragraph.

As follow up action, the implementation of a "second or third step type" National Control Laboratory is highly recommended. The existing facilities, with modifications, at the Institute ONAPHARM seem to be the best among those inspected in Yaoundé.

Short description of ONAPHARM's present and planned activity.

ONAPHARM /Office National Pharmaceutique/ became operative in 1985/1986, directed by Dr. Bobo Beye, assisted by a WHO expert.

The main duty of the Institute is to organize the supply of medicaments in the country. For this reason, ONAPHARM orders, stores and distributes a number of medicines supplying the country continuously. /Organigramme in Annex 5./. As a decision of an interministerial meeting held in Yaoundé on 19-22 July 1988. ONAPHARM has to be specialized also for the quality control of medicaments applied in Camerron, and later of those used in the UDEAC countries. It is also planned to introduce the production of some medicaments, first from bulk, later from raw materials.

Situated in the centre of Yaoundé, ONAPHARM has a rather steep area with a number of buildings used mainly for storage of medicaments.

For the above expansion of ONAPHARM's activity, there is the project "Prohealth" of the Government, supported financially by WHO, assisted technically by experts of Ciba-Geigy /Bruxelles and Basel/. In the frame of this project, remodelling of some buildings is in process, where the quality control of human vaccines at national level has to be considered. From this point of view, the following possibilities are to be taken into consideration.

1. The building for the chemical and physical quality control of the medicaments is already reconstructed by chinese experts, in very good quality. /Annexes 6, 7/. According to previous estimates made by international experts, in the first stage, beginning probably in 1990, 300 analyses are to be completed per year, approximatively.
   A number of the equipment has arrived in 1988, while others will arrive in 1989. The training of the personnel is planned for 1989. Here, on a territory of more than 160 m^2, a number of tests required for human vaccine control could also be performed /pH, adjuvant content, preservative content, residual moisture etc./.

2. Close to the above building, an other one is also reconstructed for the storage of solvents, consisting of two rooms /Annex 8./ According to the opinion of the director of ONAPHARM, this building could also be a nucleus for vaccine control, and the storage of the solvents could be made elsewhere.

3. A building, the same size as that for chemical control, will be reconstructed in 1989 for microbiological, immunological and other purposes, planned by chinese experts. /Annex 9/. Here, sterile room shall be available. The room planned for immunological investigations could be used for the control of human vaccines, too.

4. There are plans for the remodelling of a small building for animal housing. /Annex 10/. In the case of the introduction of human vaccine control /potency and safety tests/, a bigger

house seems to be necessary for the laboratory animals /mice, guinea-pigs, rabbits/. For this reason, the remodelling of a bigger, existing building, about 60 m^2, at present used for nothing, is recommended.

Summerizing the possibilities regarding the estaulishment of the national control of human vaccines, the Government has to decide the level of control to be introduced. Beside the appointment of a National Control Authority /or Technical Adviser/, the implementation of a National Control Laboratory in the frame of ONAPHARM is recommended. The direction of ONAPHARM agrees with this imagination. The control of imported and locally produced /LANAVET/ human vaccines could be carried out here.
It seems to be advantageous, that the development of LANAVET and ONAPHARM occurs in parallelisme. A rather quick governmental decision is required on this matter, as further reconstruction acitivities at ONAPHARM depend on whether human vaccine quality control has to be done here or not.

In the case of a positive governmental decision, the preparation of a detailed Project Document concerning the introduction of National Control of Vaccines in Cameroon is recommended.

E. Registration

As there is no human medicament and vaccine production in the country at all, the registration procedure is unknown in that form required in developed countries. Imported products require a "visa" by the Ministry of Health before distribution.

As the production of pharmaceutical preparations /medicaments and vaccines/ will be introduced in the near future, regulations of their registration have to be elaborated, taking in account international experiences.
Registration procedures might be studied abroad and assisted locally by international experts.

TOIRE VETERINAIRE

DE

AROUA BOKLE

BATIMENTS

RATS - SOURIS

G.B. 20

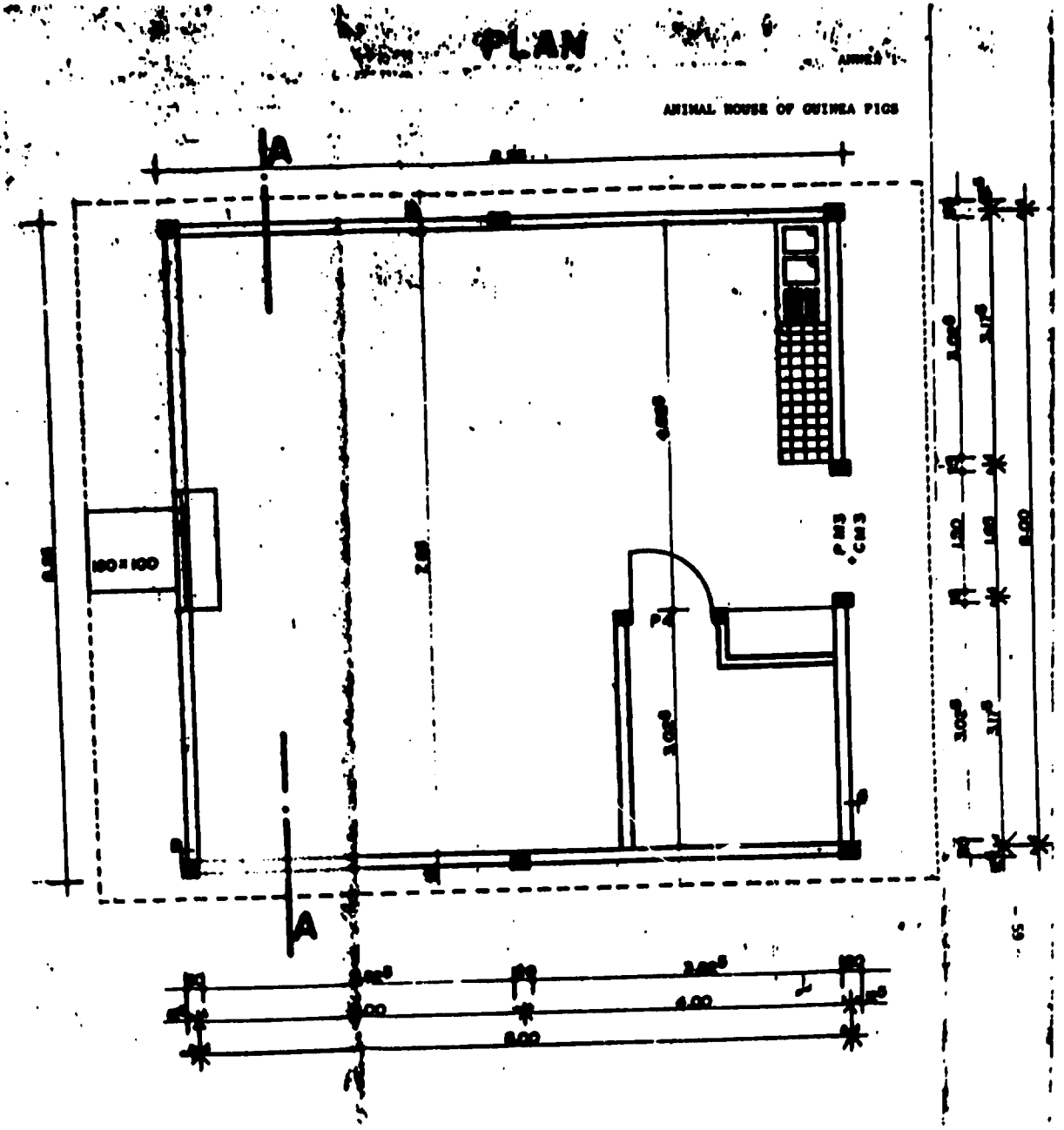
DATES		DATES	MODIFICATIONS

T E

PLAN

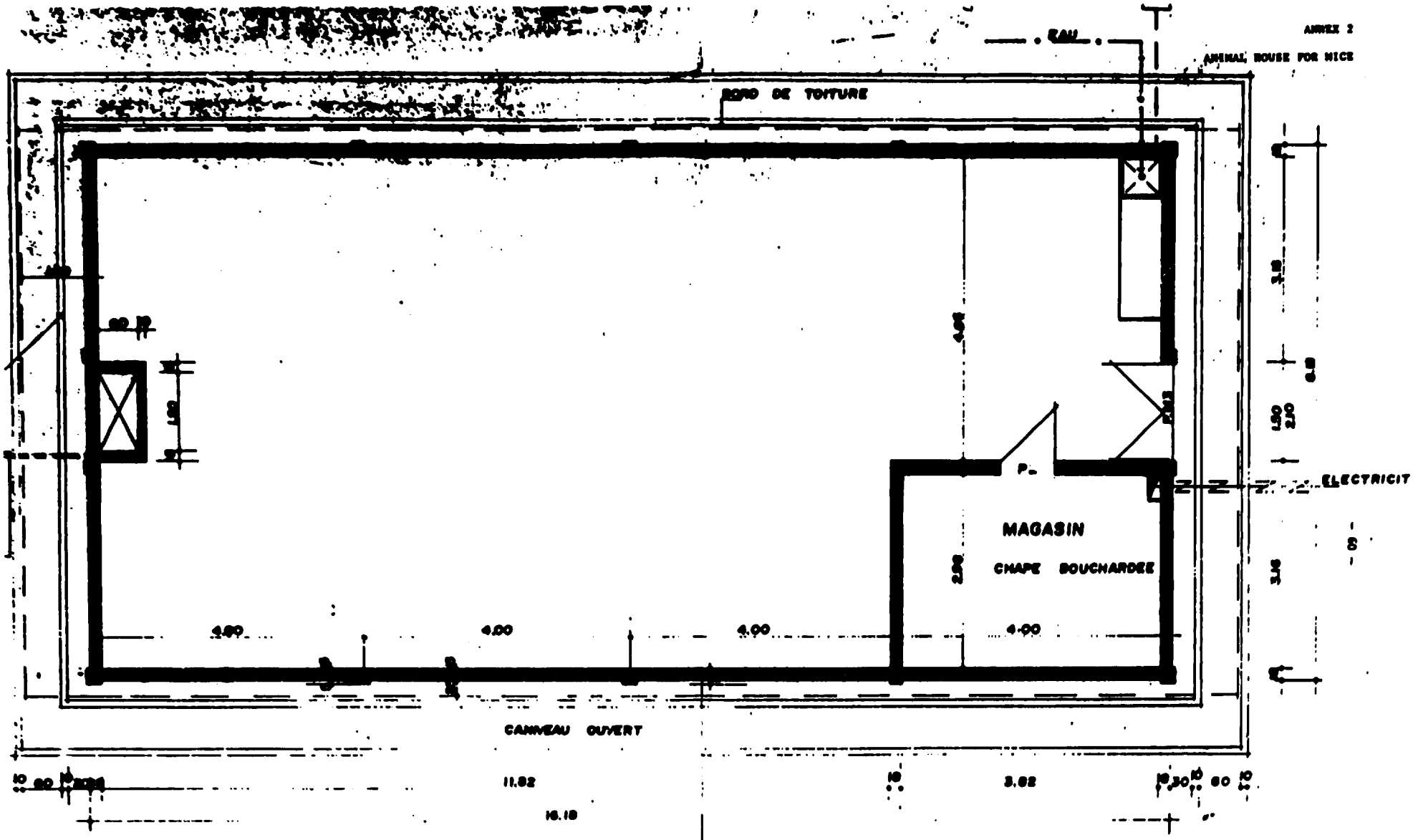
ANNEXE

ANIMAL HOUSE OF GUINEA PIGS

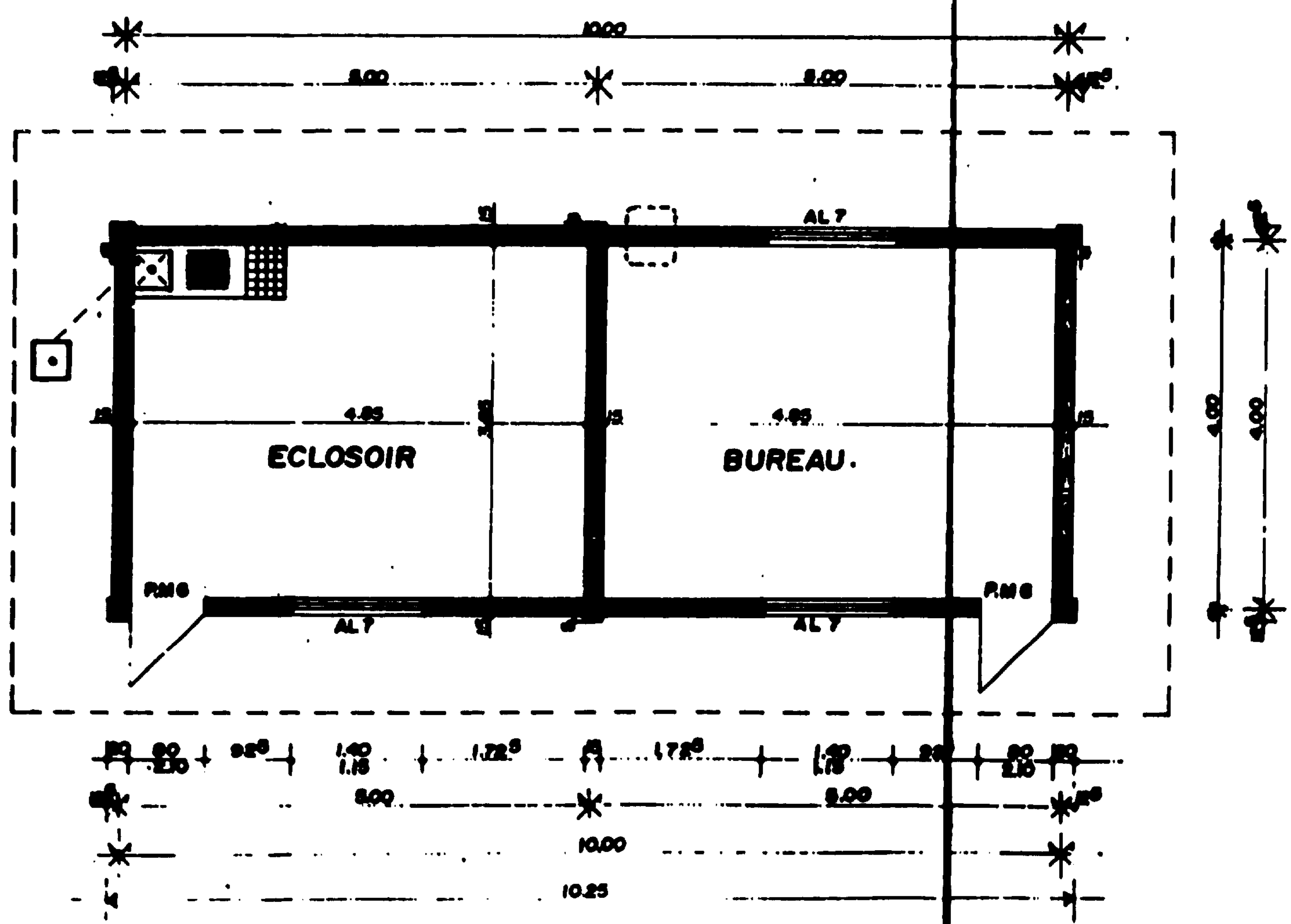


ANNEX 2

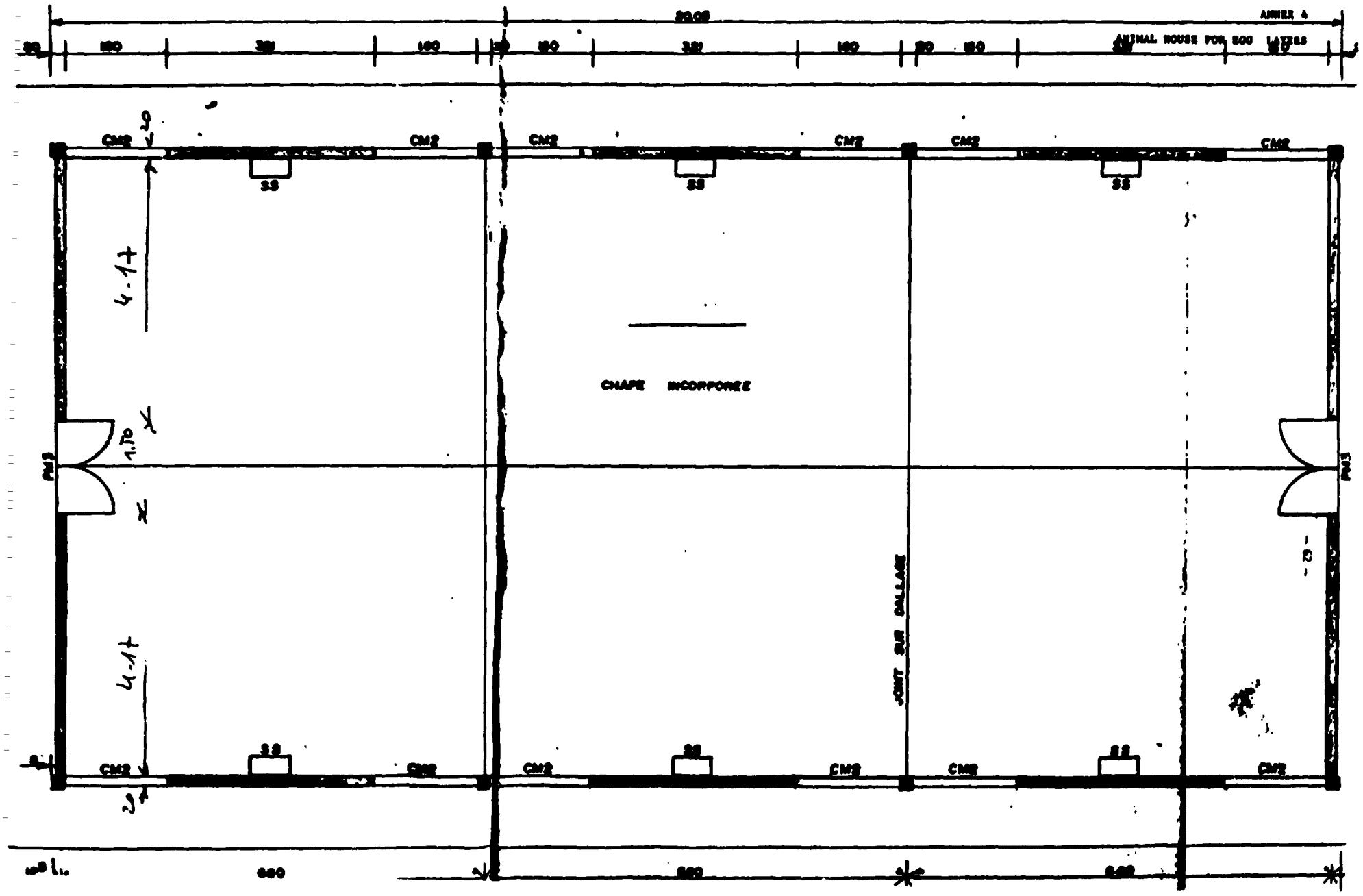
ANIMAL HOUSE FOR NICE



PLAN

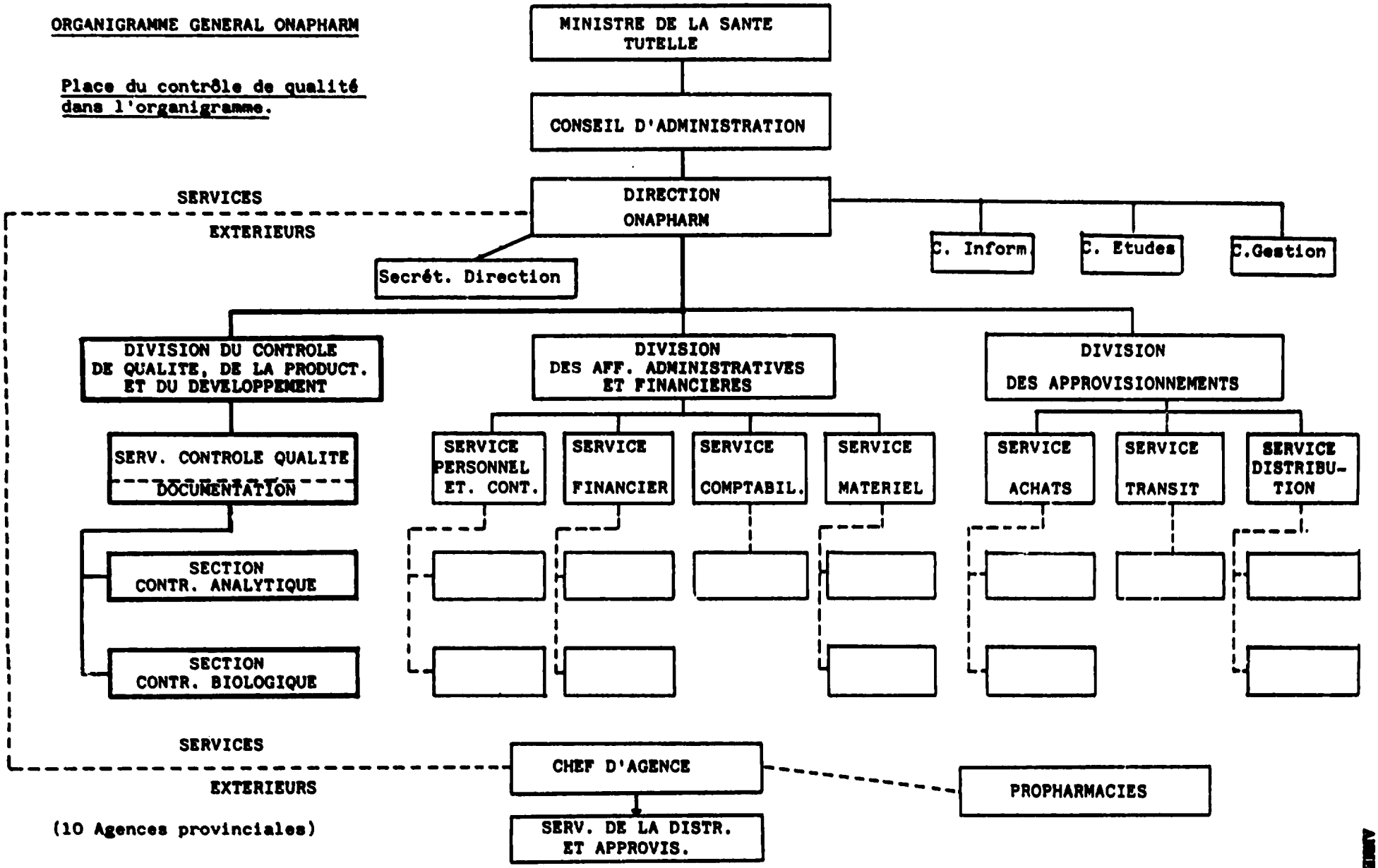


10.00
5.00
5.00
ECLOSOIR
BUREAU.
4.85
4.85
AL 7
AL 7
AL 7
P.M 6
P.M 6
4.00
4.00
10.00
10.25


ANNEX 4
ANIMAL HOUSE FOR 800 LAYERS
CM2
SS
4.17
CHAPE INCORPOREE
1.70
PU3
JOINT SUR DALLAGE
4.17
CM2
SS
CM2

ORGANIGRAMME GENERAL ONAPHARM

Place du contrôle de qualité dans l'organigramme.



(10 Agences provinciales)

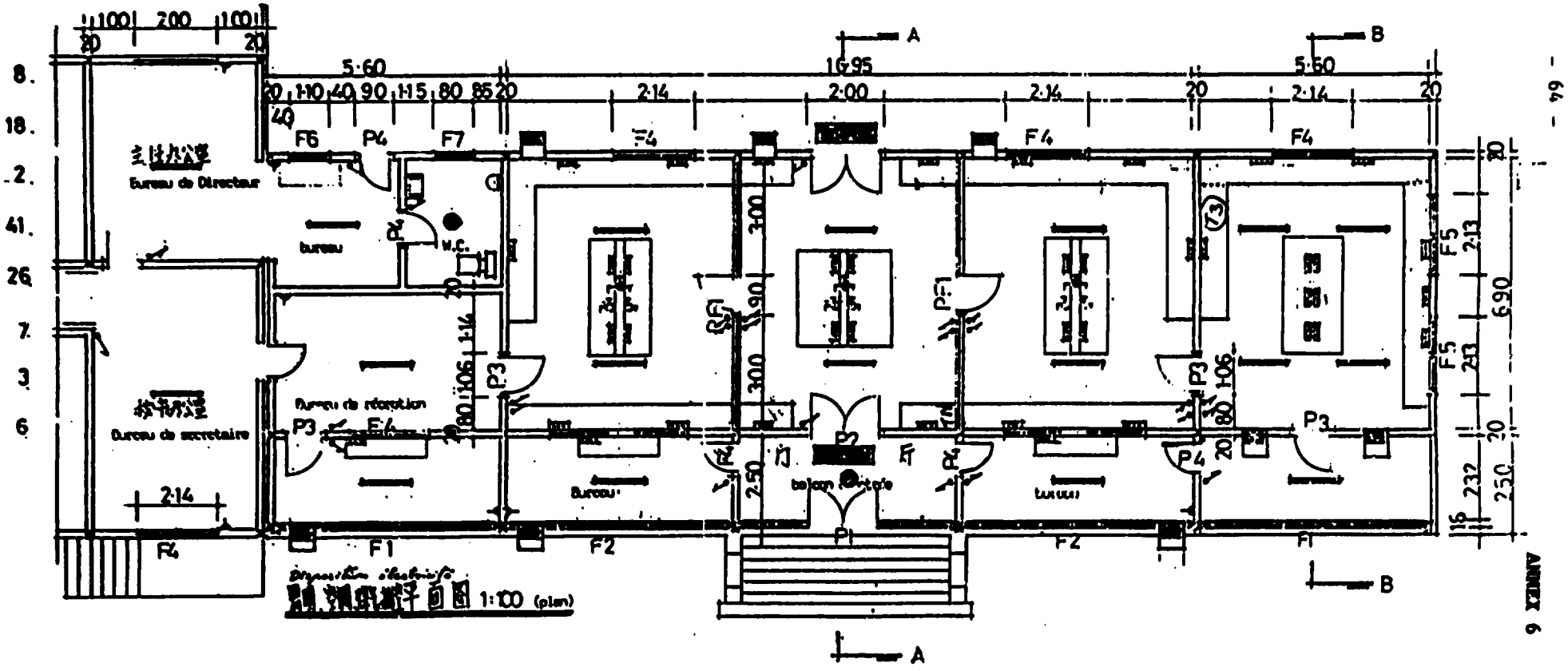
ANNEX 5

Equipement électrique : prises de courant, éclairage

Equipement gaz

Equipement vide

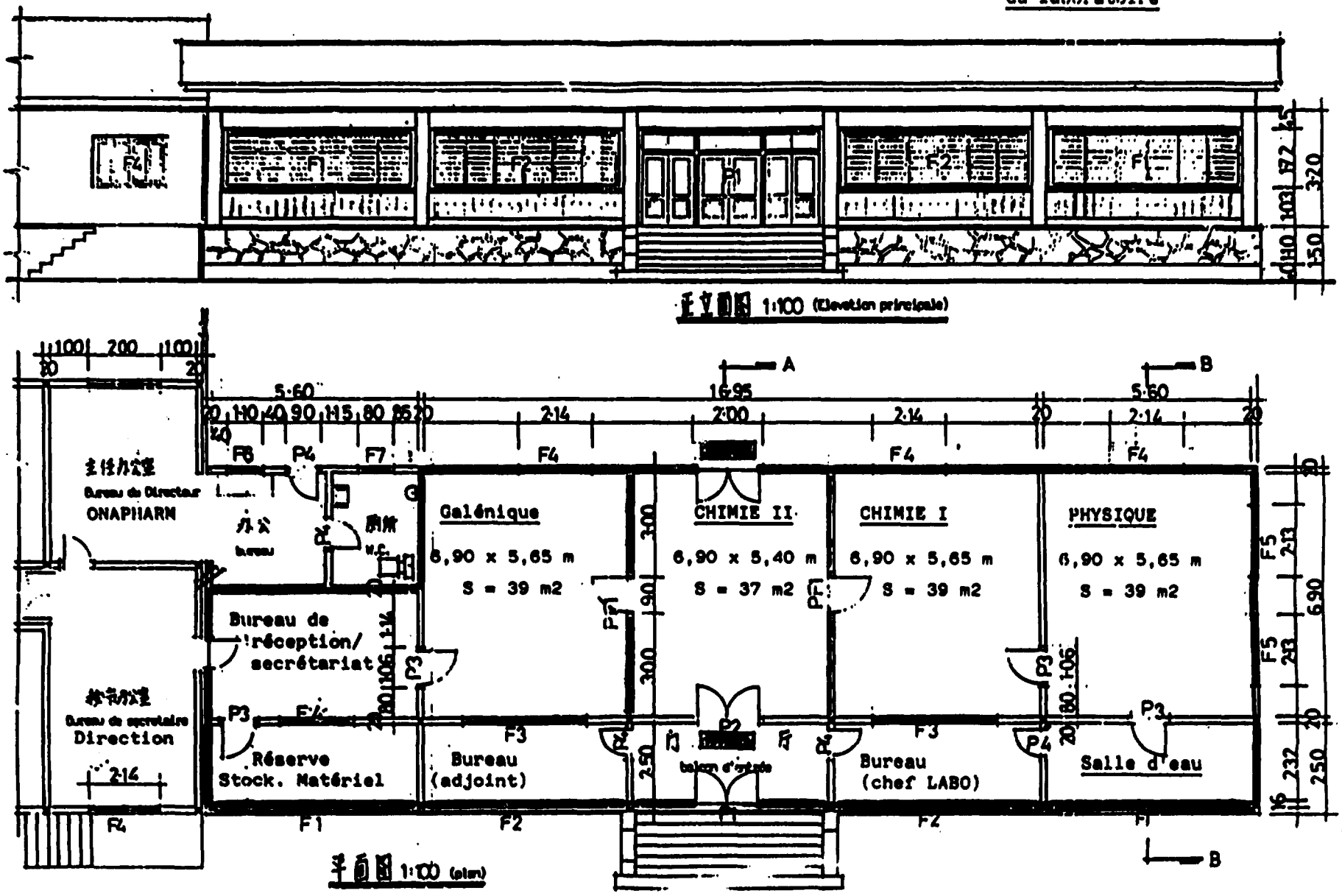
Climatisation



ANNEX 6

sique + galénique + chimie I + CHIMIE II = 154 m2

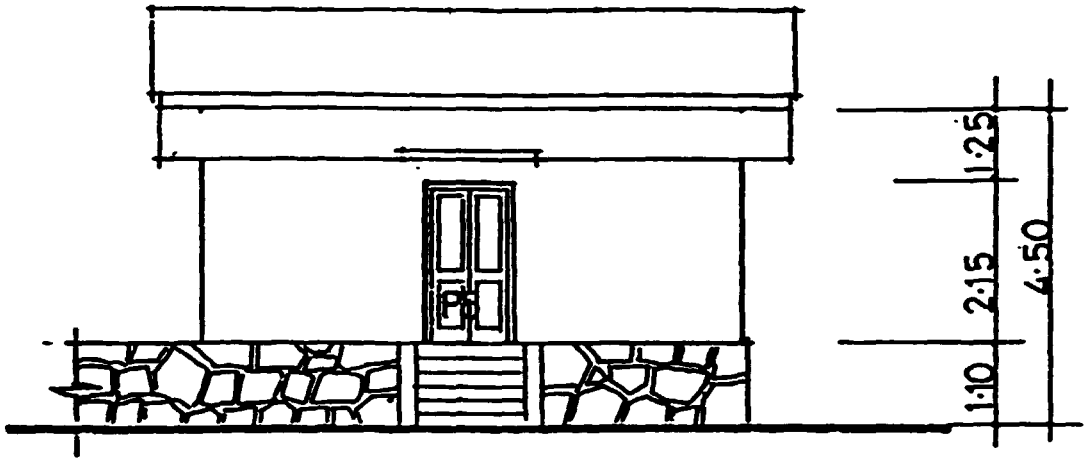
Affectation des divers locaux du laboratoire



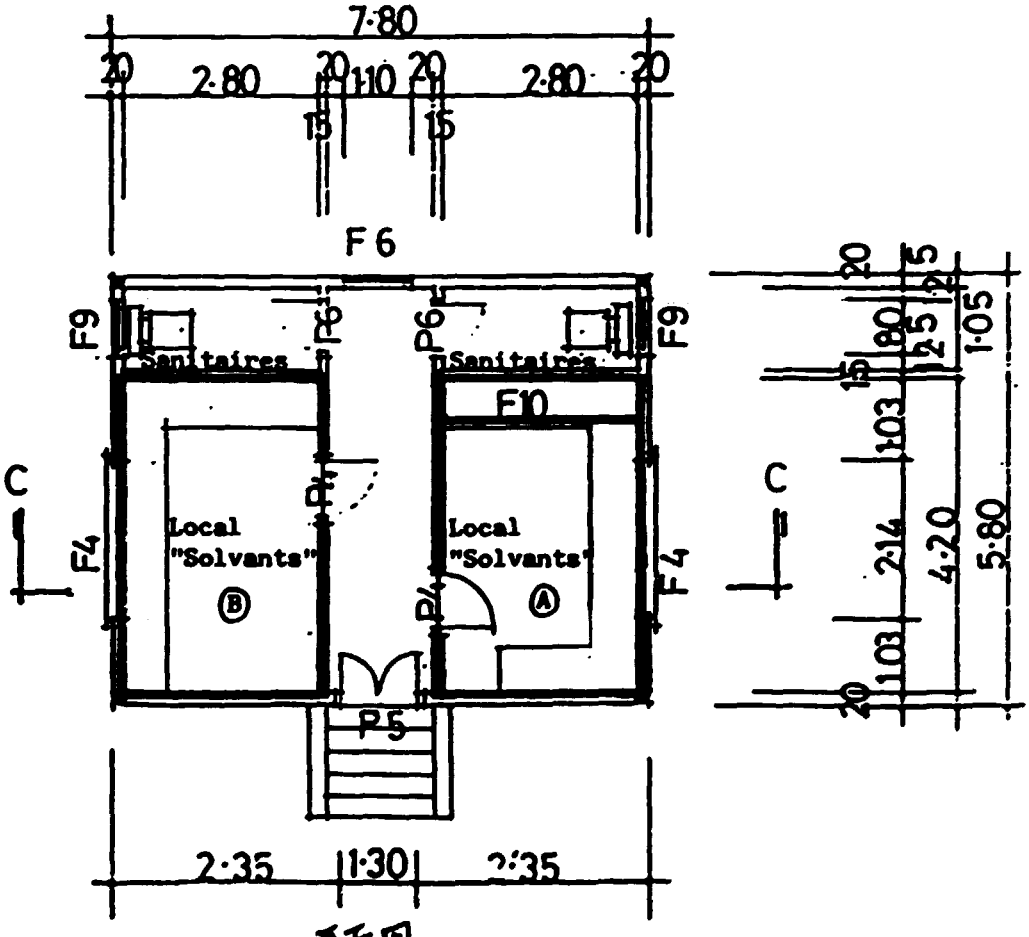
ANNEX 7

PLAN DES LOCAUX

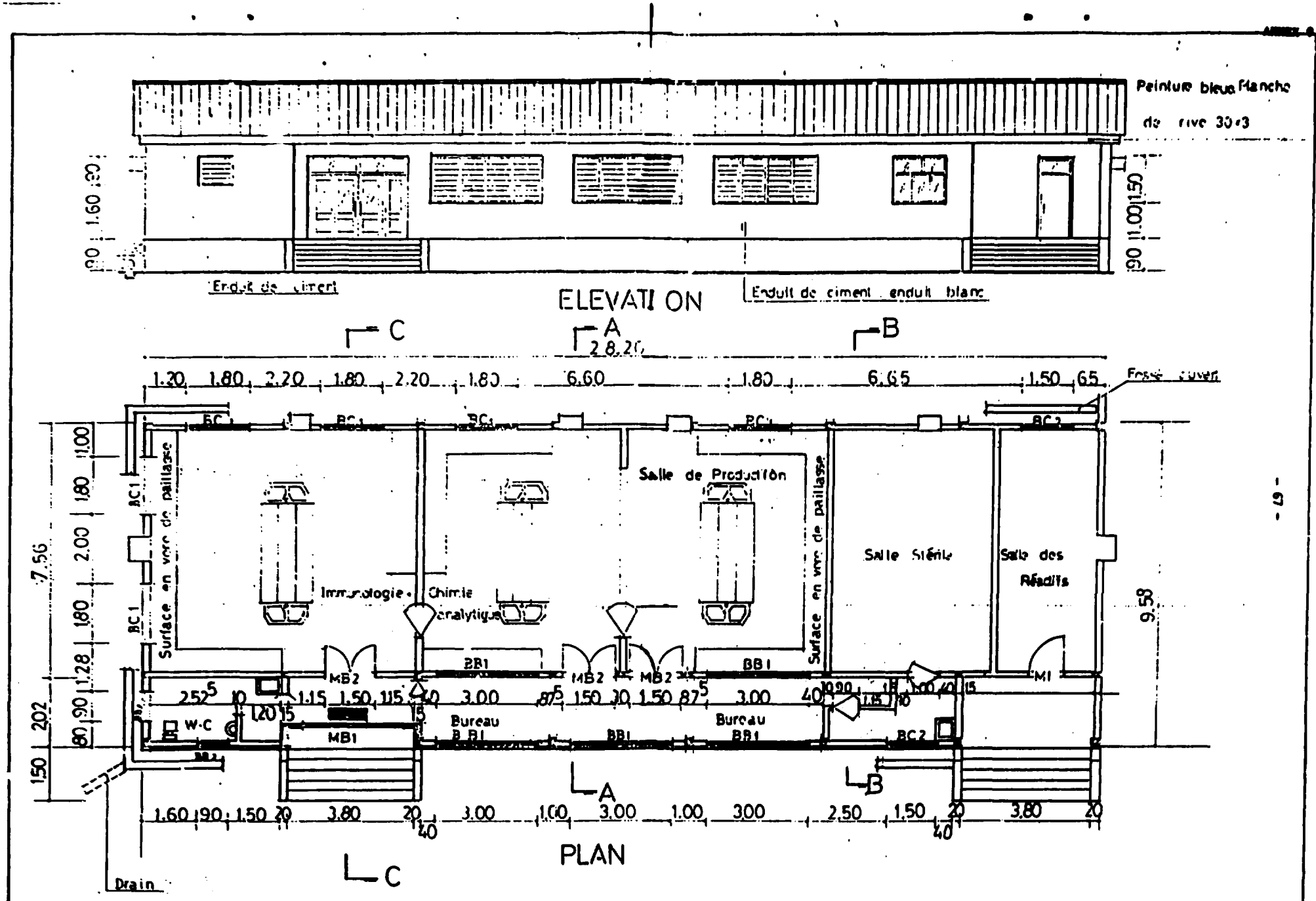
"STOCKAGE SOLVANTS"

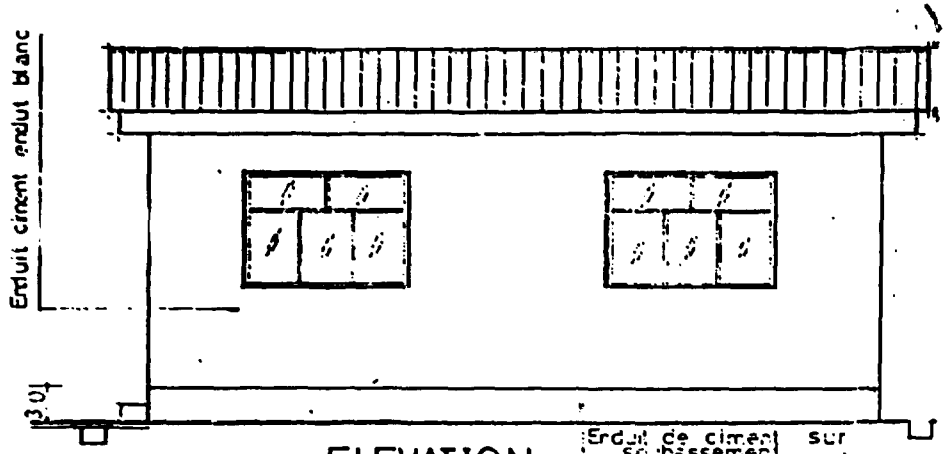


正立面图 1:100

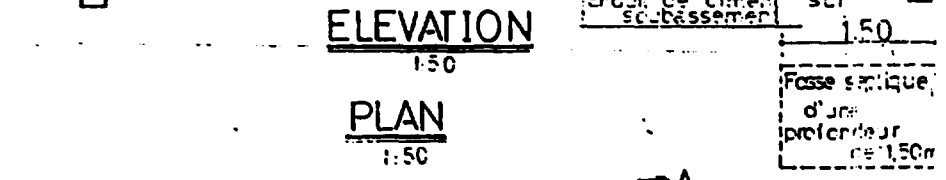


平面图 1:100

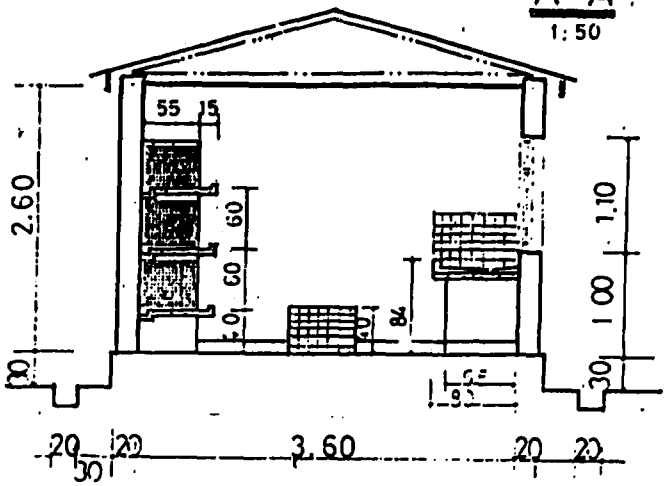

Peinture bleue Planche de rive 30x3
Enduit de ciment
Enduit de ciment enduit blanc
ELEVATION
PLAN
Salle de Production
Immunologie
Chimie analytique
Salle Stérile
Salle des Réactifs
Bureau
W.C
Drain
Surface en vrai de paillasse
Frise cuivre
A
B
C



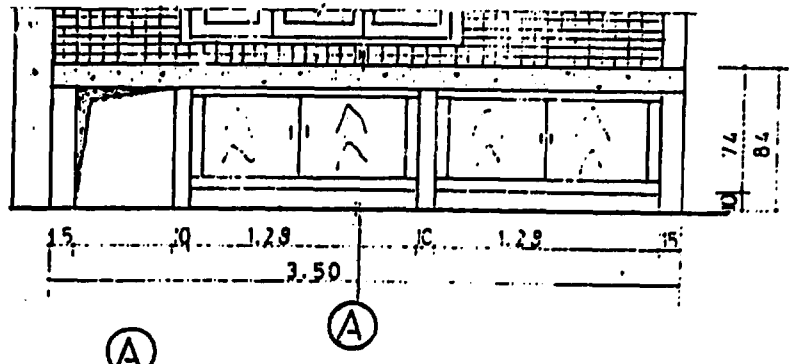
ELEVATION
1:50

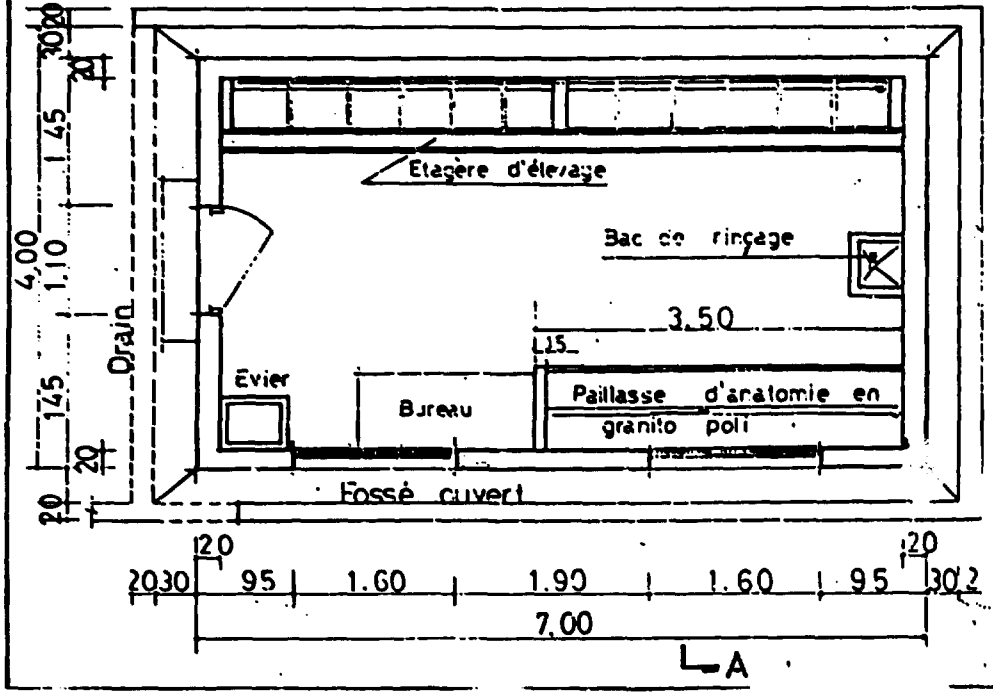


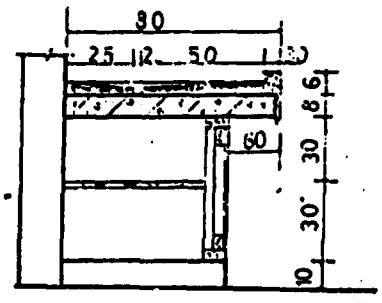
PLAN
1:50



A–A
1:50







PLAN ET ELEVATION
DE
SALLE D'ANIMALERIE