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MEDICAL RESEARCH IN PARIS

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1944

COMBINED INTELLIGENCE OBJECTIVES
SUB-COMMITTEE

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MEDICAL RESEARCH IN PARIS
5 September 1944.

REPORTED BY

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CIOS TARGET NUMBERS.
24/18(a),24/18(b), 24/19, 24/19(a)
Medical

Allied Forces. Supreme Headquarters

COMBINED INTELLIGENCE OBJECTIVES SUB-COMMITTEE
G-2 Division,SHAEF (Rear), APO 413.

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Allied Forces. Supreme Headquarters

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PERSONNEL OF TEAM

William A. Howard, Lt. Col., MC., USA, HQ ETOUSA, (Captain).
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A.E. Francis, Lt. Col., RAMC, Everleigh Vaccine Laboratory.
Arthur P. Long, Lt. Col., MC, USA, HQ. ETOUSA.
Hamilton Southworth, MD, OSRD, U.S. Embassy, London.
L.L. Waters, MD, OSRD, U.S. Embassy, London.

Report of Medical Investigating Team.

INTRODUCTION

Official targets of the Medical Investigating Team were:-

a. Pasteur Institute, No. 28 Rue du Docteur Roux - 24/18a
b. Pasteur Institute, Garches. - 24/18b
c. Union Intersyndicale, 44 Rue du Colisees.
d. Distillerie de Deux-Sevres, Melle. - 24/19
e. Rhone-Poulenc Laboratories. - 24/19a

I Pasteur Institute

The largest and most important of the targets was the Pasteur Institute. The main portion of the Institute is on the Rue du Docteur Roux, while the section at Garches deals primarily with the production of serums and toxoids, chiefly those for the prevention and treatment of diphtheria and tetanus. The director of the Institute is a Doctor of Physical Sciences, Dr. Jacques Trefouel, while the director of that part of the Institute located at Garches is Dr. Gaston Ramon. All work in the Institute is supervised by chiefs of sections, each of whom has a special field of endeavor. The functions and accomplishments of the Institute can best be described by considering individual subjects which were investigated rather than by describing each department or section.

a. Penicillin.

(1) The Pasteur Institute up to the present time has been preparing only small amounts of penicillin sufficient to meet its own small needs for therapeutic purposes. Penicillin is produced from a single strain of penicillium, namely P. notatum--4222 (Fleming). The Rhone-Poulenc Laboratories are using the same strain, which was furnished to them by the Pasteur Institute. During the five weeks prior to this investigation, no penicillin had been manufactured, largely due to the lack of supplies, culture media, etc. Before this time the production of penicillin was approximately 400,000 units per week. A program had been devised to increase this output to approximately 500 grams per week, the material having a strength of 60 to 100 units per milligram. This would increase the output to between 30 million and 50 million units per week. Dr. Nitti has succeeded in preparing a few gramsof penicillin which he claims has a strength of 8,000 units per milligram. The amounts of penicillin presently being prepared in the Institute are insignificant in comparison with the quantities manufactured in the United States and Great Britain.

(2) P. notatum is grown only on surface cultures and the liquor has a strength of approximately 20 units of penicillin per cc.

(3) The material produced has been used principally in staphylococcal and pneumococcal types of infection, a total of 30 patients having been treated. Penicillin has also been used in ophthalmic

infections both by direct application to the conjunctiva and by sub-conjunctival injection.

(4) Penicillium corylophilum DX is a strain which was obtained from Holland and which has been used by Dr. Levaditi. This strain differs from Penicillium notatum in that it does not produce penicillin. The substance which is produced is called corylophillin by Levaditi. It is active in vitro but has no action in vivo. It is not toxic to animals even in large doses. This substance does not appear to have any therapeutic value. A culture of Penicillium corylophilum DX was obtained by Col. Francis.

b. Typhus.

(1) The section on typhus of the Pasteur Institute is under the direction of Dr. Giroud. The typhus laboratory is an integral part of that portion of the Institute on the Rue du Docteur Roux.

(2) Dr. Giroud maintains two strains of classic typhus virus; one is the Tunis strain, which has already been made available to experimenters in this field in the United States and Great Britain; the other is a strain obtained from the Warsaw epidemic of 1941. Guinea pigs infected with these two strains have been taken to London by Major T. E. Woodward of the United States of America Typhus Commission. In Dr. Giroud's laboratory, the seed strains are maintained by weekly passage through rabbits.

(3) The vaccine produced is the Durand-Giroud rabbit lung vaccine. The total production of the Laboratory is approximately 300 li ers of vaccine per month. This amount is prepared by the inoculation of 20 rabbits daily for four days of each week. At the present time vaccine also is being prepared from dog lungs. The potency of the vaccine is measured by rickettsial agglutination and by the Giroud virus neutralization test. The vaccine is not washed but is centrifuged. The expiration date of the vaccine is ordinarily set at 12 months after its preparation although Dr. Giroud feels that it is effective up to two or three years. The vaccine is an effective antigen, probably more satisfactory than the early Cox type vaccine but not as potent as the type presently produced in the United States. Dr. Giroud does not employ eggs in the production of vaccine. The Germans used approximately 10 per cent of Dr. Giroud's monthly output of typhus vaccine.

(4) New Techniques.

(a) Dr. Giroud has developed a diagnostic rickettsial slide agglutination test using rabbit lung suspension as an antigen. This is a rapid method and is said to be quite satisfactory.

(b) Dr. Giroud uses the intradermal technique in rabbit skin to demonstrate neutralizing antibodies.

(c) He feels that there is a hypersensitivity in

individuals convalescent from typhus fever which can be demonstrated by the production of an intradermal reaction after injection of formalized antigen. It was stated that this could be demonstrated two years after recovery from typhus.

(d) Dr. Giroud feels that freezing the rickettsial virus over long periods actually steps up its antigenicity.

c. Diphtheria.

(1) Toxin. Diphtheria toxin is produced in media prepared from a papain digest of horse meat or from the Martin digest. From the former a toxin is secured which averages 60 l.f. units per cc. and from the latter 50 l.f. units per cc. The strain of C. diphtheriae used in toxin production is the Park 8 strain. It is the feeling of Dr. Ramon and other members of the Institute staff that this strain is satisfactory against both the so-called "gravis" and "mitis" forms of diphtheria. The total output of toxin has been increased from 80 liters to 600 liters per week since the beginning of the war. The major portion of toxin produced is used in the manufacture of toxoid (anatoxine) for the immunization of horses in the production of diphtheria antitoxin.

(2) Toxoid. Toxoid (anatoxine) is prepared in the usual manner by the use of 0.6 per cent formalin, and incubation for approximately 30 days at 40 degrees centigrade. This amount of formalin is somewhat higher than that used by the United States and British manufacturers. It was stated that the extra amount was required for toxins produced either on papain digest or Martin digest media. Once detoxified, reversion to toxicity has never been experienced. Diphtheria toxoid is manufactured separately but for administration it is bottled in combination with tetanus toxoid and triple typhoid vaccine. Dr. Ramon feels that the use of this "melange" adds to the antigenicity of the individual antigens and therefore does away with the necessity of adding an agent such as alum.

(3) Antitoxin. A concentrated variety of antitoxin was prepared prior to the war, using the sodium sulfate fractional precipitation method. Due to lack of supplies in the last few years, only unconcentrated antitoxin is now prepared. Three strengths are available: 300 units per cc., 500 units per cc. and 1000 units per cc.

(4) All materials produced by the Laboratory were used for the French with the exception of such antitoxin as was prepared from German loaned horses. The Germans wanted to obtain toxoid and antitoxin from the Laboratory in bulk to resell in smaller units under German labels. The Institute was able to forestall this plan by convincing the Germans that there would be considerable danger of sabotage by French workers if they realized that all the materials were going to Germany. As a consequence almost the entire output of antitoxin was used by the French.

(5) Combined Therapy. The recommended method for the treatment of diphtheria is a single dose of antitoxin not to exceed 40,000 units, with the simultaneous administration of one cc. of toxoid subcutaneously. This to to be followed at weekly intervals by increasing doses of toxoid.

d. Tetanus.

(1) Toxin. Tetanus toxin is prepared in essentially the same media as diphtheria. The toxin obtained contains a minimum of 20 l.f. units per cc. and 50,000 guinea pig m.l.d.'s per cc. with a maximum of 200,000 m.l.d. Dr. Ramon states that the l.f. value is as reliable a measure of toxicity in tetanus toxin as it is in diphtheria toxin.

(2) Toxoid. The toxin is detoxified in the same manner as for diphtheria. The resulting toxoid will flocculate satisfactorily, as does the toxin, but it falls off 10 to 20 per cent in l.f. value.

(3) The recommended method of administration is in 3 doses at 7 to 10 day intervals, givin in combination with diphtheria and triple typhoid. A stimulating dose of 1 cc. of tetanus toxoid alone is given one year later and subsequently at the time of injury, at which time Dr. Ramon feels there is no indication for the administration of antitoxin. Dr. Ramon reports that there are no reactions of sensitivity to toxoid prepared on papain digest media. Reactions to the combined diphtheria, tetanus, triple typhoid "melange" are stated not to occur in infants and only "moderate" reactions are encountered in older children and adults.

(4) Unconcentrated tetanus antitoxin is prepared as in the case of diphtheria. Strengths available are: 300 units per cc., 1000 units per cc. and 2000 units per cc. Dr. Ramon stresses the combined sero-anatoxine therapy for tetanus as in the case of diphtheria.

e. Bacterial Vaccines.

(1) Because of the shortage of meat, autolysed yeast is now used to supply certain essential components of bacteriological media. All vaccine cultures are heat-killed and no preservative is added. Strains used are chosen from those which have been freshly isolated from active cases of various diseases. These strains are studied for virulence and completeness of anti-genicity before they are adopted for use in the preparation of vaccines.

(2) Triple Typhoid Vaccine. The bacterial composition of triple typhoid vaccine per cubic centimeter is as follows: -

Eberthella typhi	466 million organisms.
Salmonella paratyphi	200 million organisms.
Salmonella schottmuelleri	333 million organisms.

(3) Cholera. This vaccine is prepared from strains received from Indo-China. These strains are similary studied for virulence and antigenicity. The vaccine contains 4 billion organisms per cc. Two injections are given at 7 to 10 day intervals, the first 1 cc. and the second 2 cc.

(4) Plague. For some time a vaccine was produced from Bacillus pseudotuberculosis of rodents, which was thought to give cross protection for Bacillus pestis. This preparation has been discarded in favor of a vaccine devised by Dr. Girand of Madagascar. This consists of an attentenuated strain of living plague bacilli, probably in a manner similar to Otten's vaccine.

(5) Staphylococcus. Both staphylococcus vaccine and staphylococcus toxoid are prepared but are not in demand.

(6) Streptococcus. No streptococcus toxins or antitoxins are prepared.

(7) Dysentery. The Institute does not prepare a dysentery vaccine.

(8) Meningitis. Meningococcus anti-serum, "Types A & B," are prepared but are not considered important.

(9) Influenza. No influenza virus vaccine is prepared.

(10) Pneumonia. There is no pneumonia antiserum or vaccine. A small amount of serum is prepared for typing purposes.

(11) Miscellaneous. The following antitoxins or antiserums are prepared:-

(a) Shiga antitoxin.
(b) Anthrax antiserum.
(c) Botulinus antitoxin, Types A & B.
(d) Antivenin (against vipers, European and African, and cobras).

f. Gas Gangrene

(1) An unconcentrated gas gangrene antitoxin is prepared with the following formula: -

	Parts by volume
Perfringens	4
Septique	4
Histolyticus	1
Oedematiens	2
Sporogenes	1

The univalent serums from which this combination is prepared, each have a strength of 100 units per cc. The material is packaged in 10 cc. ampules, two of which are considered necessary as a prophylactic dose. For treatment 12 to 15 ampules are needed. By the standards of the United States and Great Britain, this antitoxin appears to be extremely weak. No gas gangrene toxoid for human use has been produced.

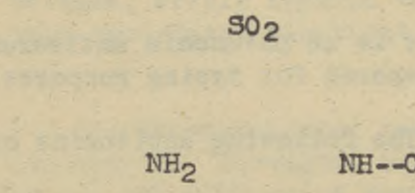
(g). Rabies Vaccine.

(1) The Pasteur Institute uses 4, 3 and 2 day-old cords, which have been dried and preserved in glycerol for not longer than 8 days prior to use. The Institute also produces a carbolized brain vaccine for use in the French Colonies. If refrigerated, this last product may be kept for 3 to 6 months.

(h). Sulfonamides and related compounds.

(1) Officials of the Institute recommend the use of sulfanilamide locally and claim great success with this drug. Sulfanilamide is preferred because of its solubility. Sulfamerazine is used for oral administration, especially because of its ease of manufacture.

(2) One new promising sulfone (succinyl-diphenyl-sulfone) has been developed and studied at the Institute. This substance is highly soluble and forms a neutral solution. The formula is given below:-



This compound is stated to be better than sodium sulfacetamida (German albucid soluble). The lethal dose of this drug is 50 milligrams for a 20 gram mouse (2.5 grams per kilo.) Succinyl-diphenyl-sulfone is not as good as the other sulfonamide drugs orally because it is not as cyanosis. It is suitable for local use and for use in the conjuntival sac.

(3) Dr. Nitti of the Institute staff has been engaged in work connected with anti-sulfonamide activity.

(i) Preservation of Milk

(1) Prof. Dr. Bertrand has developed a substance which when added to unpasteurized milk will keep it sweet for 8 to 10 days. This substance may be eliminated by boiling. The nature of this new material was not revealed but it is expected that the commercial product will be called "Microlysine."

(j) Laboratory Equipment.

(1) Five items of equipment are considered to be worthy of special mention. These are as follows:-

(a) Ultracentrifuge. This has been built by Dr. Pierre Lepine and is patterned after the model at the Rockefeller Institute. It is used principally for virus research.

(b) Microforge. This is an instrument designed by Dr. Ramon and his staff for the manufacture of very small instruments and material from glass, etc. with this instrument it is possible to make capillary tubes with less than a micron internal diameter.

(c) Micro-manipulator, which is a pneumatically controlled device for dissection and manipulation in the microscopic field.

(d) An apparatus for cinemicrography.

(e) Microbiophotometer. This is an ingenious instrument for the continual automatic recording of the growth rates of bacteria as measured by the turbidity of suspensions. Recordings are made by means of a commercial recording thermograph used in conjunction with a photoelectric cell.

k. Personalities of the Pasteur Institute

(1) Dr. Jacques Trefouel, Director of Pasteur Institute.

(2) Dr. Pierre Lepine, Director of Virus Research

(3) Dr. Gaston Ramon, Director of Pasteur Institute at Garches.

(4) Dr. F. Nitti, Chief of the Section on Chemotherapy.

(5) Dr. Bonnefoi, Chief of the Vaccine Service.

(6) Dr. Lafaille, Chief of the Diphtheria Service.

(7) Prof. Dr. Bertrand, Chief of Physiology Section.

(8) Dr. Giroud, Chief of the Typhus Service.

(9) Mademoiselle Guillaumie, Chief of the Gas Gangrene Service.

(10) Madam Th. Trefouel, Chemist, and wife of the Director, who collaborates with him in some of his research work.

1. Officials of the Pasteur Institute stated that the present supplies of biologicals are adequate for the needs of France and that the principal problem is one of distribution rather than of manufacture. It is evident, however, that there is a critical shortage of some materials essential to the manufacture of certain products of the Institute.

II. Union Intersyndicals.

a. According to Dr. Trefouel this organization no longer exists and during its period of activity was only a wholesale trading company. Dr. Trefouel suggested that a visit be paid to Pharmacien General Finnel for information concerning the manufacture of antimalarials. From M. Finnel it was discovered that the bulk of such work was done by the Rhone-Poulenc Laboratories.

III. Rhone-Poulenc Laboratories.

a. The Rhone-Poulenc Laboratories are a part of the firm known as "Specia". M. Billon is Director General of this organization. The Director of the Rhone-Poulenc Laboratories is M. Bo. Prof. Paul is the Scientific Director of the Rhone-Poulenc Laboratories and is assited by Dr. Decourt.

b. Since 1935 Rhone-Poulenc has made products under a license for the I.G. Farbenindustrie. No original work on 4-amino quinolines has been done. The Laboratory was asked by the I.G. Farbenindustrie to experiment with quinacrine (atabrine) and plasmoquin. The Rhone-Poulenc group combined the I.G. Farbenindustrie products to form a compound sold commercially as Premaline. This combination is stated to be satisfactory and the dosagea employed are approximately the same as ordinarily prescribed for these two drugs when used singly.

c. Sontoquino.

(1) In 1941 the I.G. Farbenindustrie suggested that Rhone-Poulenc carry out trials with a new drug known as Sontoqui ne. Three forms were described, known respectively as Sontoquine M (methane-Bisoxy-Naphthoate), Sontoquine C (hydrochloride), and Sontoquine R (resorcin carbonate). Sontoquine M is the least soluble and apparently is most suitable for suppression since it gives a longer action. The curative doese of Sontoquine M is stated to be 0.6 grams daily for 5 days. It is well tolerated with no side effects, such as gastrointestinal symptoms or discoloration of the skin. Sontoquine C and Sontoquine R are soluble and act more rapidly. Dosage of these two drugs is 0.3 grams daily for 5 days administered in a manner similar to that of atabrine. An injectionable solution is prepared known as Sontoquine soluble. This gives slight local reactions and has no particular advantages over the oral type. Sontoquine is active against schizonts and has only slight action against gametes. Hyperpyrexia is usually controlled in 12 to 36 hours. For therapy, it appears that Sontoquine C is better tolerated than atabrine and is more effective than Sontoquine R. Sontoquine M is preferred for suppression and Sontoquine C for treatment.

Field trials were conducted in North Africa, but were interrupted by the German collapse. It was believed that there are fewer relapses than with quinine and that relapses were rare with adequate treatment. The Germans have obtained these results from the Rhone-Poulenc Laboratories. I.G. Farbenindustrie have not been experimenting with any new products which have been revealed to this group of investigators.

d. The material prepared from cultures of Penicillium corylophilum DX appears to be notatin since it cannot be extracted by amyl acetate. Up to 8 months ago the Germans had not made any penicillin. At the present time experiments are apparently being conducted by Dr. WIndhaus of Gottingen and by Ciba, the Swiss pharmaceutical firm, which is working on a laboratory scale. A complete review of the subject of penicillin appears in the Schweitzer Medizinische Wochenschrift; Vol. 74: No.23, page 611, under date of 10 June 1944.

IV. Distillerie de Deux-Sevres.

a. This target is located in Melle in the department of Deux-Sevres, north and east of Bordeaux, and does not properly come under the activities of this team. Information concerning its activities was sought from the Director of the Pasteur Institute, who stated that so far as he knew their work was concerned with moulds and ferments and their antibiotic activity. He did not know of any significant investigations by this group.

V. The Institute National d' Hygiène.

a. This is an organization which was founded in Marseille in 1940 under the auspices of the Rockefeller Foundation and with the assistance of Dr. George K. Strode and Dr. John B. Youmans. The function of this organization is primarily to stimulate research by suggesting problems and furnishing material assistance to investigators. It is concerned principally with administration and the coordination of activities. The Director is Dr. Chevallier, whose present headquarters is in Paris at 45 Rue Cardinet. No further investigation of this organization was made.

VI. Pasteur Institut, 28 Rue du Remorqueur, Brussels.

The Pasteur Institut is located about 200 yards from the end of Rue due Remorqueur where it joins the Rue Wiertz. There are two storeys above the ground floor, and attics. Each floor is divided into 4-5 rooms, all of which are laboratories, and open into a common corridor opposite Rue du Remorqueur. Storerooms are located in the basement. The building is surrounded by shrubbery and trees and faces Parc Leopold. The director of the Institut is Docteur Paul Burdet, son of the former director, Docteur Jules Bordet, now retired. All work is supervised by the Director.

The number of personnel on the staff was stated to be more than adequate. As a means of preventing many young Belgian scientists from being transferred to Germany for work there, the Institut had

practically doubled its personnel during the period of German occupation and this accounted for the large staff.

Very little investigation work has been done at this institution since the time of the German occupation. In fact one might say that medical research as such ceased at that time and was replaced by work of a practical nature. This change was instituted, not by desire but by urgency as it became necessary for the institution to prepare (a) large quantities of vaccines and sera, (b) to examine all throat cultures for diphtheria (25,000 positive cultures were recorded each year), (c) to prepare all sera for blood typing, and (d) to examine all blood collected by the Belgian Red Cross for syphilis (20,000 positive blood tests per year were recorded).

The individual subjects which were investigated are here listed.

(a) Penicillin

No strains of penicillin had been made available to the Institut, and henceno studies had been made.

(b) Malaria and other Tropical Diseases

Malaria, tropical diseases, louse-borne and allied diseases are neither treated nor studied at the Pasteur Institut, Brussels. This work is carried out at the "Medicin Tropical", Antwerp, under the direction of Professor Rodhains, former Chief of Tropical Diseases in the Belgian Congo.

(c) Typhus

Only a small amount of typhus vaccine is produced at this laboratory. The strains employed and methods of production are similar to those used at the Pasteur Institut, Paris.

(d) Tetanus

Tetanus toxin, toxoid (anatoxine and antitoxin are prepared in the manner recommended by Dr. Gaston Ramon, Directeur, Pasteur Institut, Garches.

(e) Diphtheria

About 1,000 litres of toxoid (anatoxine) and 3,000 litres of antitoxin have been produced each week since the beginning of the war. The strains employed and method of production are similar to those employed by Dr. Gaston Ramon. 52 horses were supplied to the institution by the Germans for the preparation of antitoxins for German use. 74 additional horses were purchased by the Institut from Belgian peasants for the production of antitoxins for use by the Pasteur Institut.

(f). Bacterial Vaccines

(1) Typhoid vaccine is prepared in the usual manner, but consists of four strains rather than the usual three. These strains are B. Typhosus, Para A, Para B and Para C. The incidence of typhoid in Belgium is very low.

(2) Anthrax vaccine. Due to the low incidence of anthrax a vaccine is not prepared.

(3) Staphylococcus, Streptococcus; Dysentery, Cholera and Plague vaccines are not prepared as there is no demand for them.

(4) Pneumococcus Vaccine and Antiserum. There was such a low incidence of pneumococcal infection that neither a vaccine nor an antiserum was made.

(5) Meningococcus Antiserum. Not produced.

(6) Gas Gangrene Antitoxin. This is produced in small quantities only. The following organisms are employed in its production: Perfringens, Septique, Histolyticus, Oedematiens and Sporogens.

(g). Sulfonamides and Related Compounds.

(1) A large supply of sulfonamide is on hand. This agent has been employed for clinical use only.

(2) No original research has been made with this agent, nor have its derivatives been employed.

(h). Insecticides

No studies with these agents have been made, nor new insecticides developed.

(i). German Stimulants

It was known by the Belgians that the Germans were employing an agent to combat fatigue and exhaustion among their troops. The substance was given by mouth in tablet form and was very effective. According to Dr. Jules Bordet, it bore the name of "Actedaon". The composition of this substance was unknown to the workers at the Pasteur Institut. The investigator was unable to obtain a sample of this preparation for subsequent study and analysis.

(j). Sea Sickness and Air Sickness

No studies on the subject have been made, nor were any contemplated.

(k). Treatment of Burns; Blood Substitutes.

No studies on these problems have been made and none were contemplated.

7. The director of the Pasteur Institut stated that there were sufficient biolobicals on hand to meet the demands of Belgium adequately. Dr. Bordet added that the institution was badly in need of: (a) certain materials for the production of biologicals, (b) motor transportation for these agents, and (c) medicals literature. It is of interest to note that the most recent medical journals in their library were published in 1939-1940.

R. Cannon Eley,
Commander (MC) USNR