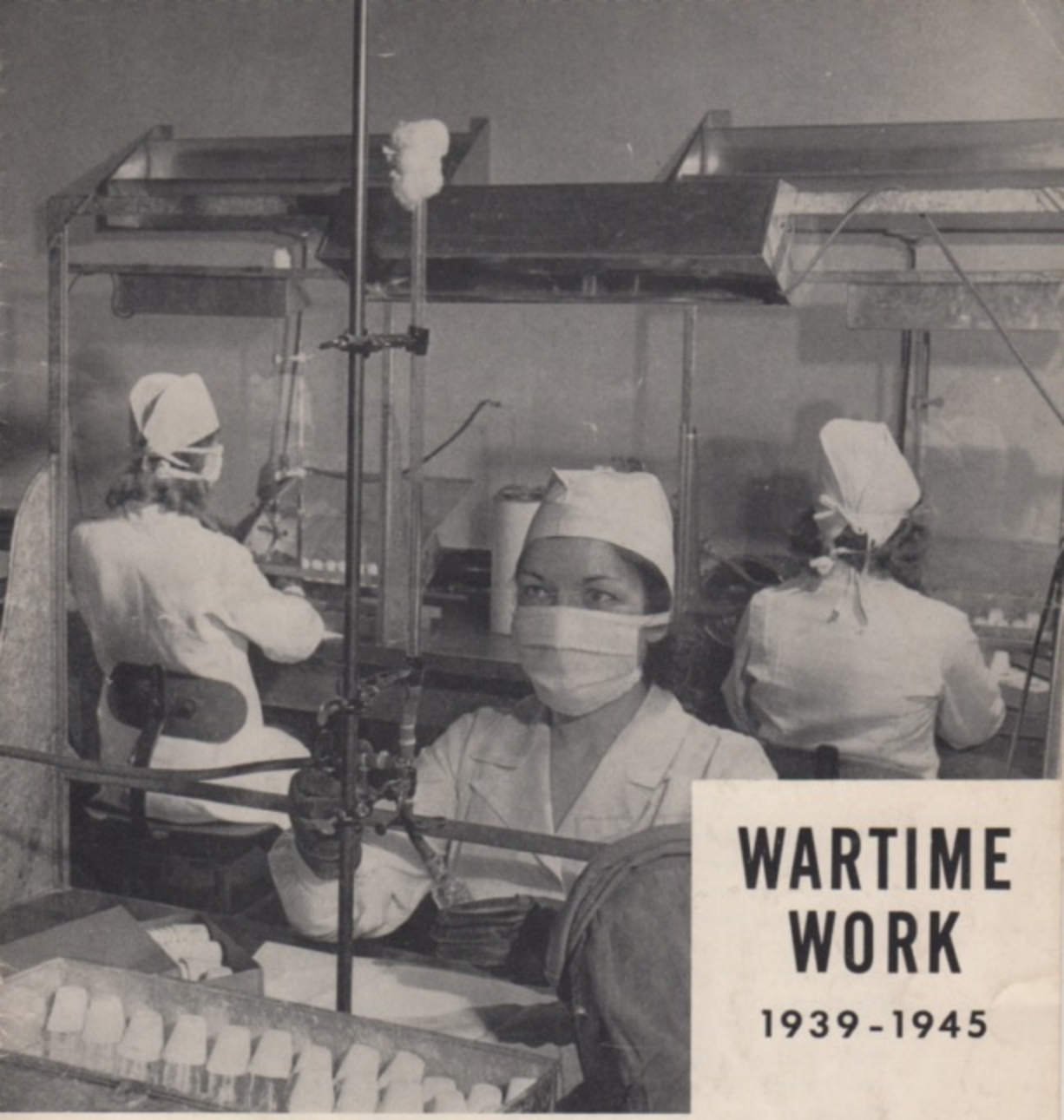

WARTIME WORK

1939-1945

CONNAUGHT MEDICAL RESEARCH LABORATORIES
UNIVERSITY OF TORONTO

THE CONNAUGHT MEDICAL RESEARCH LABORATORIES DURING THE SECOND WORLD WAR, 1939-1945

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THE Connaught Medical Research Laboratories, University of Toronto, were established for the advancement of preventive medicine and public health through research and through the preparation of biological products essential in the prevention and treatment of certain diseases. The Laboratories render a medical public service to all parts of Canada and, to an extent, to countries abroad. Their work was initiated and developed by the late Dr. J. G. FitzGerald, the first Director of the Laboratories, who, in 1914, undertook the preparation of diphtheria antitoxin in the Department of Hygiene, University of Toronto, in an effort to facilitate reduction in the toll of deaths from diphtheria in Canada. At the same time he conducted investigations into this and other diseases. These investigations were the beginning of researches in preventive medicine which have kept pace with the growth of the Connaught Medical Research Laboratories—more than sixty research studies being now in progress in the Laboratories.

Within a few months of the commencement of the work of the Laboratories, the outbreak of the First World War occurred. Soon the problem of supplies of anti-tetanus serum for the prevention of lock-jaw among the wounded became acute, and early in 1915 the Laboratories undertook to prepare this antitoxic serum. Funds to facilitate this particular undertaking were provided by the Canadian government, and keen interest in the Laboratories as a national service organization quickly developed. In consequence Dr. Robert D. Defries became associated with Dr. FitzGerald in the Laboratories; and the late Colonel Sir Albert Gooderham purchased the farm property which is now part of the Dufferin Division of the Laboratories. There Colonel Gooderham erected a laboratory building, stables and superintendent's residence, and then donated the whole property to the University for the use of the Laboratories. Thus became possible the preparation and supply by the Laboratories of all the tetanus antitoxin required for the Canadian forces in the First World War, together with large amounts of other biological products including anti-meningococcus serum and smallpox vaccine.

This experience stood the Laboratories in good stead when the Second World War broke out and brought heavy demands for many of the products regularly prepared by the Laboratories. On this account preparations such as smallpox vaccine, typhoid-paratyphoid vaccine, and diphtheria toxoid were

produced in large quantities for the armed services. There was also intensive activity in the development of new products such as typhus vaccine, penicillin, and dried blood serum, as well as in the modification of existing processes and facilities to permit increased production of established preparations. The extent of such activities may be gauged from the fact that the number of persons employed in the Laboratories rose from 252 to approximately 900 between the commencement and the end of the war. In addition 91 employees went on active service, of whom two, Mr. John Richards and Mr. Gordon A. Wilson, gave their lives in the service of their country. Of the senior research and administrative staff of the Laboratories, four—Dr. M. H. Brown, Mr. F. Lorne Hutchison, Dr. D. L. MacLean, and Dr. N. E. McKinnon—were privileged to make notable contributions to the war effort, first in the wartime work of the Laboratories and later in active service with the Canadian Army or the Royal Canadian Air Force.

Typhus Vaccine

Typhus fever is caused by a member of a group of virus-like organisms called rickettsiae in honour of Dr. H. T. Ricketts, an American, who died in Mexico while studying the disease. When war broke out in 1939, no vaccine for general immunization of troops against this disease was available and the only typhus counter-measure was delousing. When the war ended, this situation was completely changed. The vaccine problem had been solved and the production of typhus vaccine in Canada and the U.S.A. was sufficient to meet all needs. Further, the value of D.D.T. in preventing the transmission of typhus had been established. These two weapons, typhus vaccine to immunize the individual, and D.D.T. to eradicate the vector, supplement each other.

In regard to immunization, there was evidence that a vaccine consisting of killed rickettsiae would protect persons against typhus fever. The main problem was to find a method of growing rickettsiae of the epidemic variety of the disease on a sufficiently large scale—a scale permitting the wholesale inoculation of fighting troops with vaccine. In 1938 Dr. Herald R. Cox, of the United States Public Health Service, had shown that rickettsiae of the murine type of typhus fever could be readily grown in fertile, developing hen eggs if inoculated into the yolk sac. Although relatively good growths of these rickettsiae could be obtained without difficulty, Cox had initially to persevere with the epidemic typhus strain for six months before he obtained sufficiently good cultures to show the organisms under the microscope. Later, much better but somewhat irregular results were obtained. Early in 1940 Zinsser, Plotz and Enders, working in the Harvard School of Public Health, developed a method of growing the rickettsiae of epidemic typhus on chick-embryo tissue kept alive on a solid nutrient medium in flasks, and they reported their belief that considerable quantities of vaccine might be produced in this way.

It was felt at this time by the Connaught Medical Research Laboratories that an endeavour should be made to develop the production of a typhus vaccine in Canada. Dr. James Craigie undertook this work in the Labora-

tories and the National Research Council of Canada made a grant in support. Dr. Craigie then visited and worked in Professor Zinsser's laboratory in Boston. Following his return in July, 1940, he spent five months in a comparative study of the Cox and Zinsser methods of growing typhus rickettsiae. By the end of 1940 the method of Cox seemed the more promising. Two problems, however, had to be solved. Much richer cultures were required, and some adequate method of separating the organisms from yolk and yolk-sac tissue had to be found. Increasingly richer cultures were obtained by utilizing information gained in the course of a detailed study of the growth habits of the rickettsiae. The problem of purification was solved by taking advantage of a property of the yolk present in the crude vaccine preparation. Egg yolk contains substances which aid in forming emulsions. Ether, which is relatively insoluble in water, can be emulsified in water to which a little egg-yolk has been added. The suspended globules of ether attract yolk granules and tissue fragments to their surface, but rickettsiae and other micro-organisms are repelled from this surface. This, in brief, is the principle on which the method developed by Dr. Craigie was based.

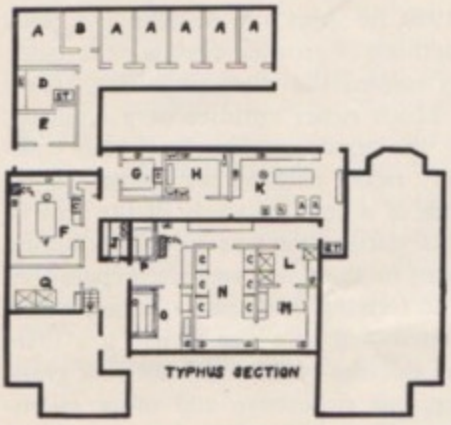
The production of typhus vaccine on a large scale was organized in two stages—culture and processing. The first stage was carried on under the supervision of Dr. Laurella McClelland at the Dufferin Division of the Laboratories and begun in August 1942. Most valuable aid was given by Mr. M. D. Orr in the organization of this work. At the peak of production 2,000 fertile eggs were inoculated each working day. The inoculated eggs were incubated for eight to ten days until the developing embryos died of typhus. The yolk sacs were removed and reduced to a fine state of division by grinding in saline; and the living rickettsiae were then inactivated by formalin. An efficient system of purifying the vaccine by the ether method was organized and supervised by Dr. Raymond Parker in the College Division of the Laboratories. After purification the vaccine was refrigerated in bulk pending the necessary sterility and potency tests.

Preparation of typhus vaccine involved translating results of research work into terms of mass production. Dr. Dennis W. Watson, previously of the University of Wisconsin, was a member of the typhus research group from September 1942 to December 1943 and made a number of valuable studies, in co-operation with the other members, which resulted in increasing greatly the extent of the yolk-sac infection and the potency of the final vaccines.

A preparation of killed micro-organisms does not necessarily constitute an effective vaccine against the corresponding disease. The substances which stimulate immunity may be destroyed or lost in preparing the vaccine. The choice and design of the potency test for typhus vaccine was a most important research problem. The development of the test used in Toronto required a great deal of exacting work in which Miss E. M. Clark and Dr. John Crawley most ably assisted. The test followed the development of special methods and differed from that used elsewhere.

By the autumn of 1942 the main and essential steps in the mass production of typhus vaccine had been determined. Certain modifications were made

TYPHUS FEVER RESEARCH AND VACCINE PRODUCTION



The interior of the main laboratory building (right) which was altered to provide for typhus fever research and vaccine production.

Special laboratory for the safe handling of eggs inoculated with typhus fever.

Some of the many shipping cases of finished typhus vaccine sent to the Armed Forces in various parts of the world.

during the ensuing year as experience increased, but thereafter the procedures remained substantially unaltered. From January 1943 the production of vaccine began to rise steadily and continued rising until the summer of 1945. During the peak of production one million doses of vaccine were being made each month. In all, more than ten million doses were supplied.

In common with typhus vaccine produced in the U.S.A., the vaccine produced by the Connaught Medical Research Laboratories arose out of Cox's discovery in 1938 that rickettsiae could be grown in the yolk sac of living embryonated eggs. Although the rickettsiae for the preparation of the vaccine were obtained by Cox's method, i.e., from yolk-sac cultures, all the details of the vaccine production in Toronto were based on independent research carried out by Dr. Craigie and his associates with the aid of grants from the National Research Council of Canada.

Tetanus Toxoid and "TABT"

In the First World War the only specific means for preventing lock-jaw among the wounded was the administration of tetanus antitoxin. The protection afforded by this serum was of short duration. Between the two world wars, a great advance was made in the prevention of lockjaw through the use of tetanus toxoid, a preparation which was introduced by Ramon, working in the Pasteur Institute, Paris. In 1923, Ramon had prepared diphtheria toxoid and subsequently had demonstrated its value in the prevention of diphtheria. His discovery of diphtheria toxoid and his later preparation of tetanus toxoid constitute two of the most important contributions ever made in preventive medicine. In the Connaught Medical Research Laboratories, diphtheria toxoid was prepared by Dr. P. J. Moloney within a few months of the publication of Ramon's results; and later, following Ramon's successful preparation of tetanus toxoid, work with this preparation was undertaken in these Laboratories by Dr. P. A. T. Sneath and continued by Mr. M. D. Orr. With this background of experience it was possible for the Laboratories to have tetanus toxoid available in quantity for the use of the armed forces early in November 1939. Experience in Great Britain, Canada, and elsewhere showed that although tetanus toxoid was markedly effective, undesirable reactions occasionally occurred following administration of the product. On this account intensive work was undertaken in the Connaught Medical Research Laboratories by Dr. Edith Taylor, who succeeded in developing an improved method for the production of tetanus toxin from which tetanus toxoid is prepared. This toxin surpassed in potency that prepared by other methods, and was of such a character that the tetanus toxoid prepared from it was free from undesirable effects.

Early in 1940 Canadian military authorities were anxious to reduce, if possible, the number of injections to be given to service personnel. Such a reduction had been achieved in the French Army, and combined vaccines, including a combination of tetanus toxoid with typhoid-paratyphoid vaccines, were in use. This experience, coupled with recommendations from the Connaught Medical Research Laboratories, led to trial experiments designed by Dr. D. T. Fraser and conducted by the Canadian Army in co-operation

with the Laboratories. As a result, a combined preparation of typhoid vaccine, paratyphoid (A & B) vaccine and tetanus toxoid—"TABT"—was prepared in the Laboratories and used by the Canadian armed forces.

During the early part of the war the method commonly in use for the testing of tetanus toxoid consisted of animal tests and laboratory flocculation tests. The animal tests were time-consuming, and the other tests were difficult to interpret, largely because of the fact that flocculation occurred in several zones. The matter of process improvements was therefore a serious problem since new preparations could not be assayed quickly with certainty. Dr. Moloney, appreciating this problem from his extensive studies with diphtheria toxoid, investigated the subject. As a result of his research, methods were developed for the preparation of tetanus antitoxin and toxoid which flocculated in single, specific zones. Using these means it became a matter of hours rather than of days to evaluate material, and thus increased production was effected.

Supervision of the production of tetanus toxoid at the Dufferin Division was one of the many responsibilities of Mr. Orr and later of Miss M. Lang. Efficient management of this work and application of the results of intensive research made possible the preparation of tetanus toxoid of remarkably high potency.

Human Blood Serum

The Canadian project for the preparation of dried blood serum for the treatment of war casualties was initiated by Professor C. H. Best, then Head of the Department of Physiological Hygiene, School of Hygiene, University of Toronto, and also at that time an Associate Director of the Connaught Laboratories. His early work in this project had the support of the National Research Council, the Department of National Defence and The Canadian Red Cross Society, and was conducted in the Department of Physiological Hygiene, with the assistance of many members of his staff including Dr. D. Y. Solandt, Dr. Jessie Ridout, Dr. R. E. Haist, Dr. A. L. Chute, Dr. J. W. Magladery, Dr. E. Fidlar and Mr. Campbell Cowan.

Blood donors were first recruited in September 1939 from members of the staffs of the School of Hygiene and the Connaught Medical Research Laboratories, and later from students at the University. An ordinary laboratory bench served in turn as a table for the assembling of equipment, a bed for blood donors, and a work bench for the processing of blood. Of necessity, all these operations were conducted in one room in the School of Hygiene building. The entire staff was composed of two or three persons who turned from one operation to another as the need arose. After blood was drawn it was typed and allowed to clot. From the clotted blood the serum was separated and the various sera were combined in groups, in accordance with their particular blood types. Subject to satisfactory sterility tests, this material was filled into 250-cc. bottles of a kind readily available. The freezing and drying operations were carried on in a small section of the School of Hygiene building, where space was provided for a freezing tray containing alcohol and solid carbon dioxide, and for a pump and drying cabinet purchased

with funds provided by the Department of National Defence. The University also made available a room in which a number of volunteer workers assisted with the project. Later, as the need of suitable accommodation for drawing blood became more pressing, facilities were made available at the former Grace Hospital near the University. Subsequently, improved facilities for a Toronto blood-donor clinic were provided by The Canadian Red Cross Society at 410 Sherbourne Street.

As the value of dried serum became evident, the work quickly expanded to the limit of the facilities of the Department of Physiological Hygiene. The success of the project made it necessary to consider re-organization in order to provide sufficient laboratory accommodation and funds. Accordingly, in October 1940, representatives of the Department of National Defence attended a meeting called by the late Sir Frederick Grant Banting, as Chairman of the National Research Council's Associate Committee on Medical Research, and by Dr. R. E. Wodehouse, then Deputy Minister of Pensions and National Health, for the purpose of co-ordinating the various groups interested in the use of human serum and of helping in the formation of an organization to ensure the production of an adequate supply. In consequence, from January 1, 1941, the project was undertaken and carried forward by the Connaught Medical Research Laboratories. Dr. A. M. Fisher directed this undertaking, and associated with him was Dr. A. F. Charles. The arrangements made with the Dominion Government through the Department of Pensions and National Health provided that the Government would reimburse the Laboratories for operational and maintenance costs directly involved in the conduct of the blood-serum work. The Laboratories undertook to contribute directorial and managerial costs, ordinary administration and overhead costs, and other indirect costs—a commitment which involved an expenditure exceeding $142,000. The Canadian Red Cross Society assumed the responsibility for collection of the very large quantities of normal human blood and serum which were required.

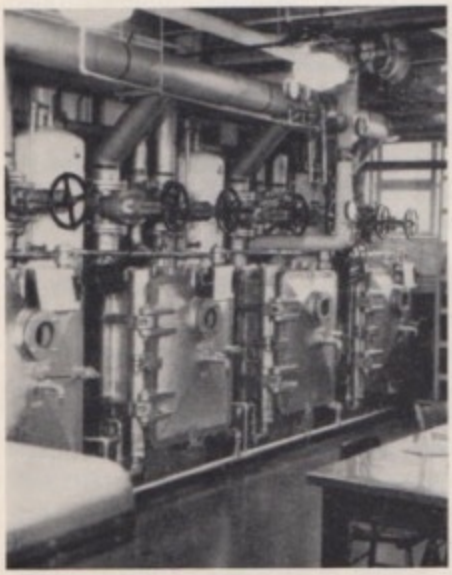
In the early days of this arrangement, clotted bloods from widely separated points in Canada were forwarded to the Connaught Medical Research Laboratories for removal of sera. As the volume of work increased, The Canadian Red Cross Society provided laboratory facilities in Halifax, Fredericton, Winnipeg, Saskatoon, Edmonton and Vancouver for preliminary separation of serum. In most instances these laboratories were organized and operated with the assistance of the universities in individual provinces. From these laboratories, sera were forwarded to the University of Toronto for completion of processing and for drying. During 1944, the last complete year in which the wartime blood-donor service was in operation, the various clinics, staffed by voluntary physicians, nurses and other interested personnel, collected 868,000 blood donations.

Operations concerned with the processing and drying of serum were originally conducted in two rooms of the School of Hygiene building. The original processing equipment consisted of one vacuum pump and a drying cabinet. Early in 1941 it was necessary to enlarge existing facilities by the purchase of three additional pumps. This was made possible by the Dominion

PRODUCTION OF NORMAL HUMAN BLOOD SERUM

A stage in the processing of serum from individual blood donations.

Storage of the donated serum in cold-rooms until processed.



A battery of vacuum dryers for drying the serum in final containers.

A typical army case containing dried serum, distilled water and administration sets.

Government through the Department of Pensions and National Health. Space for the greatly enlarged installation was provided by the Connaught Medical Research Laboratories at their Dufferin Division located a few miles north of Toronto. To accommodate the processing of the large quantities of blood being received, some peacetime operations of the Laboratories were abandoned or crowded together, and thus room was found for the preparation of blood sera and the sterilization of equipment.

During the year 1941, approximately 36,000 donations of blood and serum were received; by March 1942, the volume had increased to over 11,000 donations monthly, and further increases were contemplated. It was again necessary to increase facilities for the processing and drying of blood serum. The additional space that could be made available in the School of Hygiene building by the School and the Connaught Medical Research Laboratories, including the use of the large corridors of the building, amounted to only 3,200 square feet and was inadequate. Moreover, additional processing equipment could not be obtained for many months. It became imperative therefore to make greater use of existing space and facilities by the organization of a staff for night work. This was a major contribution to the success of the project.

At the Dufferin Division the maximum capacity for drying serum was two thousand 250-cc. bottles per week. To increase this a temporary building had to be erected in the courtyard of the School of Hygiene building, and equipment having a weekly capacity of approximately twenty-five hundred 400-cc. bottles of serum was installed there. The boiler installation for the operation of this equipment necessitated other building re-arrangements which were undertaken by the Connaught Medical Research Laboratories and made possible the completion of plans for the new serum building. By the summer of 1942, the new plant was fully engaged in the production of dried serum, with the result that in it and in the plant at the Dufferin Division there was in operation, twenty-four hours daily each day of the week, drying equipment operated by steam ejectors and four vacuum pumps, with a total weekly capacity of approximately 5,000 bottles of dried human blood serum.

By October of 1943, donations of blood and serum forwarded to the Laboratories amounted to over 57,000 monthly—an increase of 21,000 over the donations in March of the same year. It again became necessary to seek additional space, for the combined day and night staff now exceeded 140 persons. In these circumstances, and having in mind the preparation of penicillin for the armed services, the Connaught Medical Research Laboratories arranged for the purchase of the former Knox College building, on Spadina Crescent, for the purpose of two of its wartime projects—the initial steps in processing of blood serum and the preparation of penicillin. This building, which had been first a theological college and later in succession a hospital, a provincial government building, and a military headquarters, was placed in good order and, with the aid of the Dominion Government, properly equipped as a laboratory unit for the processing of more than 25,000 samples of blood weekly. Some of the operations in this work were transferred to the Spadina Division, as the new unit is now called, in January 1944, and in

April a two-day holiday in most Ontario blood-donor clinics permitted the removal of other equipment from the School of Hygiene building and its installation in the newly constructed quarters. Operations then continued on a larger scale and with increased efficiency, without a break except for Christmas Day 1944, until the cessation of hostilities.

The services provided by the Dominion Government through the co-operative arrangement with the Connaught Medical Research Laboratories extended beyond the supply of dried serum. To meet the great need for blood-typing sera in military establishments, large amounts of these sera were prepared in the Laboratories from the blood of voluntary donors. The Laboratories also provided training for laboratory personnel of the Royal Canadian Air Force in the technique of blood typing.

Although most shipments of dried serum were forwarded to England and were there packed with bottles of water necessary for dissolving the dried serum prior to use, there were several occasions on which the project of the Connaught Medical Research Laboratories was extended to include the supply of sterile pyrogen-free distilled water. Also, for the intravenous administration of any fluid appropriate apparatus consisting of hypodermic needles, drop counter, rubber tubing, etc., is necessary. In order that complete equipment might be provided, the Laboratories were asked to procure component parts which were adaptable to British or Canadian Army practice and to assemble, sterilize and package the equipment for use in the field. Thus the project grew from one having as its object the preparation of dried blood serum to an undertaking for the provision of several of the essentials in transfusion therapy. During the course of the project there was processed in the Laboratories the serum from over two million donations of blood. Shipments amounted to more than 436,000 bottles of serum, 51,000 administration sets, 34,000 bottles of pyrogen-free sterile water and 23,500 vials of typing serum. When the war ended, more than 500,000 bottles of dried serum had been processed, representing nearly 2¼ million donations of blood.

Gas Gangrene Antitoxins

About a dozen types of *clostridia* are known to cause disease in man. These organisms are widely distributed in nature because they are normal inhabitants of the intestinal tract of animals. Since under conditions unsuitable for growth these bacteria may pass into a dormant or spore stage, they may remain in the soil for long periods of time or be blown about in dust. While infections with *clostridia* are recorded from time to time, their occurrence is normally infrequent. In time of war, however, the incidence of such infections may be high and the prognosis for infected persons is grave. The most frequently infecting types of *clostridia*, as demonstrated in the Second World War, are *Cl. perfringens, oedematiens* and *vibrion septique.*

When war broke out in 1939, the Connaught Medical Research Laboratories, reviewing fields in which their functions might be of service, decided to prepare gas-gangrene antitoxins. It was possible to proceed with this work without delay because for some years, under Dr. D. T. Fraser's guidance, Dr. Helen Plummer had been studying the growth and toxin-producing powers

of *clostridia*. During the fall of 1940 and the spring of 1941, small quantities of *perfringens*, *oedematiens* and *vibrion septique* antitoxins were made. At that time, however, in the absence of large-scale military operations, and perhaps also because of lack of favourable experience in the 1914-1918 war, there was little interest in gas-gangrene antitoxins. Hence, after showing the practicability of regular production of these antitoxins, the Laboratories discontinued their production.

In May 1943, the Department of Munitions and Supply of the Dominion Government asked the Connaught Medical Research Laboratories to produce gas-gangrene antitoxins. This unexpected interest arose from the fact that during the campaign in North Africa in 1942, the British Army had found that gas-gangrene infections could be effectively treated by a combination of surgery with drugs of the sulphonamide group and antitoxin. The request was for 150,000 doses of a mixed antitoxin, each dose containing 9,000 units of *perfringens*, 9,000 units of *oedematiens*, and 4,500 units of *vibrion septique* in a volume not over 10 cc. Delivery of this amount was to commence as soon as possible and was to be completed within eighteen months from commencement of the undertaking.

Calculations based on experience gained by the Laboratories in 1940-1941 indicated that at least 300 horses would be required for the preparation of the quantity of antitoxin desired. Since the Laboratories had stabling for only about 100 horses, it was necessary to provide additional accommodation quickly. Accordingly, at the Dufferin Division, construction was begun on six wooden stables, each accommodating fifty horses, a feed storage shed, an isolation stable, an operating building, and a greatly enlarged water supply. Facilities for purification and concentration were increased approximately twelvefold by an addition to the Concentration Building. Suitable methods of large-scale purification and concentration were developed and conducted there under the direction of Dr. R. P. C. French, while Dr. Edith Taylor undertook the production of the essential toxins, in which work she was eminently successful.

The first horses were housed in the new stables in August 1943. The first shipment of antitoxin was made in October 1943, this being possible because there were on hand some of the antitoxins produced in 1940-1941. From that date onwards, the volume of shipments increased so that by August 1944 it appeared certain that the objective of supplying 150,000 doses would be achieved by the end of December. In August, however, a request came from the Department of Munitions and Supply for the Laboratories to produce another 150,000 doses, to be delivered not later than July 1945. It was realized from experience already gained that if this new task was to be accomplished, still more horses had to be obtained at once. This meant, of course, that additional stable space had to be built or found. Towards meeting this need the Hamilton Jockey Club generously agreed to rent their stables, and with this and other help a total of over 1,000 horses was maintained by Dr. E. G. Kerslake and immunized to make possible the required volume of antitoxin. In all this work Mr. M. D. Orr and Dr. P. J. Moloney made most valuable contributions.

WARTIME PRODUCTION OF GAS GANGRENE AND OTHER SERUMS

View of pump-house, 200,000-gallon water storage tank, and housing for new deep well.

Two of the nine temporary buildings, specially constructed for housing over 300 horses. In foreground, cabbages for feeding small test animals.

The concentration building, enlarged for the purification of increased quantities of serums and antitoxins required for the war effort.

It was a matter of satisfaction that gas-gangrene antitoxins were supplied to the Government on a basis which, including capital and operating costs, represented less than 50 per cent of the contract price prevailing at the time for supplies obtained from other sources by allied governments.

Influenza Vaccine

Influenza virus was first isolated by intra-nasal instillation, into ferrets, of throat washings from a patient, the virus being recovered from the turbinates of the ferret. From the ferret, the virus was adapted to infect mice, in which it produced a fatal pneumonia, and from mouse lung it was adapted to proliferate in the embryonated egg. Two antigenically different strains of influenza were early identified, and designated "A" and "B". Various substrains of A and B have since been identified, each having some minor differences from the parent A or B, but being fundamentally related.

For several years prior to the war Dr. Ronald Hare was engaged in studies in this field and established in the Connaught Medical Research Laboratories a centre in Canada for the isolation of strains of influenza virus. Thus the Laboratories were able to extend their facilities to assist in meeting the needs of the armed services relative to control of this disease.

Two discoveries that have been of particular aid in research methods were the findings that the presence of influenza virus could be identified by its ability to agglutinate red blood cells, and that chick embryo could be used for the primary isolation of virus. It had been shown that mice became immune to otherwise fatal doses of influenza virus after inoculation subcutaneously or intraperitoneally with preparations of killed virus suspensions. This led to attempts in various laboratories to prepare vaccines for use in man. The earliest vaccines were made from influenza-infected mouse lung—obviously not an ideal preparation since there was much foreign protein and always the possibility of other virus being present. Subsequently, vaccines were prepared from chick embryo tissue and from chorio-allantoic membrane, both of which, although avoiding the source of concurrent virus contamination, had a high foreign protein content and a relatively low virus content. When allantoic fluid was found to be an excellent source of virus, it was used as an immunizing agent, and better vaccines were gradually developed by concentrating and purifying the virus from this fluid.

The Connaught Medical Research Laboratories were asked to supply vaccine containing virus strains A and B for the Canadian forces. In undertaking this work, the preparation of vaccine from chick embryos was carried on under the immediate supervision of Dr. Laurella McClelland at the Dufferin Division; and the preparation of the seed virus, the testing of the vaccine and the making of pooled lots was conducted at the College Division under the control of Dr. Hare. At the Dufferin Division, Mr. Orr was responsible for the general administration of the project.

Work was commenced in August 1944 and was completed by March 1945, during which time about thirty-six members of the laboratory staff were employed in the work. This group processed about 2,000 eggs daily, preparing approximately 30,000 doses of vaccine each month.

Cholera Vaccine and Anti-Dysentery Serum (Shiga)

In view of the desire for cholera vaccine to protect members of the armed services in the Pacific and other theatres of war, fundamental work relative to the preparation of such vaccine, particularly the development of improved methods for determining its antigenic value, was undertaken by Dr. L. E. Ranta under the direction of Dr. C. E. Dolman in the Western Division of the Laboratories at The University of British Columbia. Representative cholera strains were obtained by them, and intensive study of the antigenic value of each was made in the Division. In consequence, the Laboratories were able to supply cholera vaccine for use in the armed forces. In addition, wartime studies were carried on directed towards improving the protective value of this immunizing agent.

Dr. Leone Farrell was successful in preparing anti-dysentery serum (Shiga) of high potency, and also obtained encouraging results in efforts to prepare Shiga dysentery toxoid.

Penicillin

Penicillin was discovered in 1929 by Sir Alexander Fleming of St. Mary's Hospital, London, England. This discovery provides a good example of beneficial results which may follow from careful observation of a chance happening. Working with culture plates in his laboratory, Dr. Fleming noted that some of these showed areas of inhibition of culture growth. These areas were ones where air-borne moulds had fallen on the plates. He surmised that the mould—a *penicillium*—was producing a material which inhibited growth of bacteria. After further laboratory studies he suggested that this material—penicillin—"may be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes". In 1932, Professor H. Raistrick and associates of the London School of Hygiene and Tropical Medicine contributed important information on yields of penicillin from selected media and also on its chemical extraction with ether. In 1940 and 1941 there appeared the results of a group of Oxford workers under Florey. Their report announced the preparation of penicillin as an impure, water-soluble, brown powder and showed that it was effective in treating patients. The work of this group successfully developed penicillin to an extent which fully justified Fleming's anticipations.

Following a visit of Dr. Florey to Canada and the U.S.A. in 1942, Dr. Philip Greey, assisted by Dr. A. Gray, undertook studies of penicillin in the Department of Pathology and Bacteriology, University of Toronto. Dr. C. C. Lucas and Dr. S. F. Macdonald of the Banting and Best Department of Medical Research of the University developed methods by which active penicillin could be extracted from crude broth and prepared in a form suitable for clinical use. Early in 1943 their work had progressed to a point where a pilot plant was considered desirable, and provision for this was made by the National Research Council.

In August 1943, the Dominion Government asked the Connaught Medical Research Laboratories to undertake the large-scale production of penicillin

for the needs of the Canadian armed services. Since accommodation of equipment necessary for this undertaking, and for concurrent requirements in the processing of blood serum, was not otherwise available within the University, the Laboratories arranged, as already mentioned, for the purchase of the former Knox College building and property on Spadina Crescent, Toronto. With financial assistance from the Dominion Government, this building was remodelled and equipment was installed so that in less than eight months from the time the task was undertaken penicillin was being produced there. As a result, it was possible to fulfil the initial large requirements of penicillin within the time limit specified by the Government. Production was maintained for the Government as long as special needs existed, and was continued as a regular undertaking of the Laboratories involving an extensive program of research.

At first, penicillin was produced by growing the penicillium mould on the surface of a suitable liquid medium in bottles resembling quart milk bottles. The bottles containing the culture were placed for approximately one week in rooms having special temperature-regulating devices enabling control of conditions to promote maximum growth. In an endeavour to meet the urgent need for penicillin, the penicillin laboratory was in operation twenty-four hours daily, and over 30,000 bottles were handled each day. Very special aseptic technique was required in preparing the mould spore suspensions, in inoculating the medium in the bottles, and in preventing contamination throughout the period of growth. All this called for specially developed methods and carefully trained operating personnel. Moreover, it was necessary to develop methods for the extraction of penicillin and for its purification, including the removal of impurities which would occasion reactions or pain in patients receiving injections of the product.

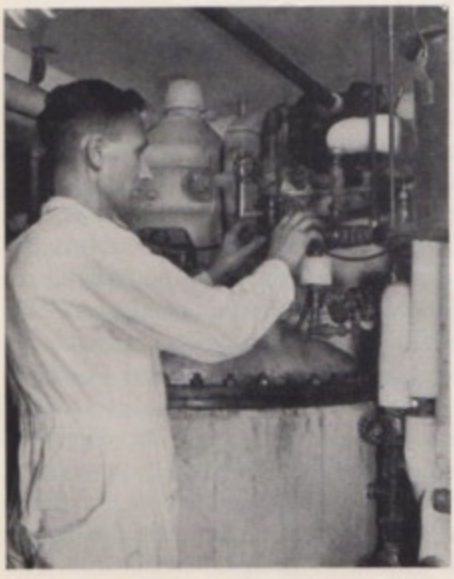
Later the "submerged-culture" process, in which deep tanks are employed, replaced the "bottle" process. Less space and labour, together with increased yields and reduced costs, were important advantages of the newer process. Intensive studies of submerged-culture methods were made by laboratories specially designated by the National Research Council of the United States, and also by several of the leading manufacturers of pharmaceuticals. Suitable mould strains for submerged growth were sought, together with determination of the necessary conditions of aeration and the best designs of tanks. The Northern Regional Research Laboratories, U.S. Department of Agriculture, selected an appropriate strain for submerged growth, and later the Carnegie Institute developed by X-ray treatment a highly successful mutant of a strain from the University of Minnesota. Then, late in 1944, plans were prepared by the Connaught Medical Research Laboratories for the production of large quantities of penicillin by the submerged-culture process. Dr. N. L. Macpherson undertook this work, and on July 1, 1945, the first tank of submerged-culture material was successfully harvested and processed. Important contributions to the successful production of penicillin were made by Mr. D. G. Dix, Dr. R. E. Carlyle, Miss H. G. M. Macmorine and Mr. A. R. Lucas.

The story of the production of penicillin in the University of Toronto is an illustration of success achieved by team-work among various departments

PENICILLIN PRODUCTION

Typical growth of penicillin in a culture bottle.

Some of the many thousand culture bottles incubated in air-conditioned rooms.



A stage in the cultivation of penicillin in deep tanks.

A laboratory worker engaged in calculating the potency of penicillin samples.

of the University. It is gratifying that not only were very large quantities of penicillin made available, but also an opportunity was provided for research with penicillin and with other antibiotics. The assistance of committees set up in Washington by the War Production Board, the National Research Council and the Department of Agriculture was extended to the Connaught Medical Research Laboratories. The allocation of essential supplies by the War Production Board, particularly items of equipment which were in very short supply, made possible the early commencement of the work. Representatives of the Laboratories were privileged to attend scientific discussions arranged by the various authorities in Washington associated with the development of penicillin production. Thus every assistance was given to the Laboratories in this work.

Much credit is due to Dr. Ronald Hare, Dr. N. L. Macpherson and Dr. P. H. Greey, who initially directed the large-scale production of penicillin in the Connaught Medical Research Laboratories, and to Dr. Macpherson for his subsequent development of production employing the submerged-culture method. Invaluable services in the penicillin program were given by Dr. S. F. Macdonald, Dr. C. C. Lucas and Dr. P. H. Greey in their capacities as consultants.

Conclusion

As is evident from the foregoing summary, much of the activity of the Connaught Medical Research Laboratories during the period 1939-1945 was directly concerned with the prevention or treatment of disease among personnel in the armed forces. But the Laboratories would have failed in their functions if they had not also continued their program of research and production in the civilian public-health field at home. As it was of national importance to prepare tetanus toxoid so it was that the production of Insulin, liver extract, heparin, diphtheria toxoid, vaccine virus and other products should be safeguarded and continued. Without the assistance and co-operation of the staff members engaged in these undertakings it would have been impossible for other members to plan and supervise the various special wartime projects of the Laboratories.

No record of the war activities of the Laboratories would be complete without acknowledgment of the counsel and assistance of Dr. Balmer Neilly, Chairman of the Connaught Committee of the Board of Governors, and of the Vice-Chairman, Mr. Frederick K. Morrow, and the other members of the Committee in coping with problems presented by the greatly increased work of the Laboratories in 1939-45.