Canad. M. A. J.
July 1, 1956, vol. 75

Special Article

# RESEARCH ON THE DEVELOPMENT OF A POLIOMYELITIS VACCINE: TORONTO, 1950-1953

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IN 1949, when Enders, Weller and Robbins of the Harvard Medical School reported that the Lansing strain of poliomyelitis virus multiplies in tissue cultures of human embryonic tissue, it immediately became possible to reopen the question of developing a poliomyelitis vaccine for human use. Earlier work of Kolmer, Brodie, and others had achieved some measure of success in this direction, but further progress was not possible because suspensions of virus could be prepared only from the central nervous system of monkeys. In view of the known occurrence of "paralytic accidents" following the use of anti-rabies vaccines prepared from central nervous tissue, it was essential to avoid such material in any vaccine to be used as a prophylactic in normal children. The work of Enders, Weller and Robbins, and that of the many other workers who quickly confirmed their original observations, pointed to a suitable alternative as a starting point for a vaccine, because the fluids of tissue cultures infected with poliomyelitis contained large quantities of virus suspended in a bland medium.

In as short a time as five years following the original discovery of Enders, a large-scale trial of a poliomyelitis vaccine pioneered by Dr. Jonas E. Salk, with the active support of the National Foundation for Infantile Paralysis, was undertaken in the United States, three Canadian provinces, and Finland. The results of this trial were satisfactory, according to Dr. T. Francis, Jr., who was entrusted with the task of evaluating the safety and prophylactic effect of the vaccine.[1]

The fact that most of the vaccine used in these trials was a joint product of the Connaught Medical Research Laboratories of the University of Toronto, and two U.S. drug houses, Eli Lilly and Parke Davis, is well-known. In an address in the fall of 1955 in Canada, Dr. Hart Van Riper, Medical Director of the National Foundation for Infantile Paralysis, stated: "... if the Connaught group had not so quickly worked out the technique for large-scale production of virus, we could not possibly have at hand today a practical vaccine for the prevention of paralytic poliomyelitis."[2]

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It is the object of this paper to record briefly some details, not previously published, of the work in Toronto which led up to the production of this vaccine. The author, as a grantee of the National Foundation for Infantile Paralysis from 1947-1953, while on the staff of the Connaught Medical Research Laboratories, had the privilege of directing some of the work.

In 1950, preliminary studies on the tissue cultivation of poliomyelitis virus were begun at the Dufferin Division of the Connaught Medical Research Laboratories by Miss M. Chapman. These experiments served to confirm the original observations of Enders. At that time, human embryos were the source of tissue, and it became desirable to move the work to a laboratory closer to the downtown hospitals. Such an opportunity presented itself in February 1951, when the work was transferred to the Virus Research Department of the newly opened Hospital for Sick Children, where space was generously made available by Dr. T. G. H. Drake, Director of Research, Dr. W. L. Donohue, Director of Pathology, and Dr. T. E. Roy, Director of Bacteriology.

In The Hospital for Sick Children, a new research staff was appointed, consisting of Dr. A. E. Franklin, Dr. W. Wood, Mrs. D. Duncan, and Miss J. Thicke. With the collaboration of Dr. Douglas Cannell and other obstetricians, an adequate supply of human embryonic tissue was made available.

Almost from the first, poliomyelitis virus was readily cultivated by the "suspended cell" or Maitland technique. Most of our experiments at that time were carried out with Lansing (Type 2) virus, and tests for infectivity were made by the inoculation of mice. Earlier studies with Miss Eina M. Clark at the Connaught Medical Research Laboratories had provided the necessary background for interpreting the results of such tests.

In 1952, it was reported by Thicke *et al.*[3] that Lansing virus multiplied in "suspended-cell" cultures of human embryonic kidney as well as in mixtures of brain and cord; monkey testis was also suitable. Furthermore, it was reported in the same paper that a chemically defined nutrient medium (Medium 199) devised by Dr. Raymond C. Parker's group, which then included Dr. Joseph F. Morgan and Miss Helen Morton, at the Connaught Laboratories, was superior to the simple balanced saline solutions in common use at that time. The composition of Medium 199 had been published in 1950.[4]

In a second paper, also published in 1952, it was pointed out that infected culture systems used much less glucose than control uninfected cultures.[5] The third report, also published in 1952, stated that Medium 199 served as an excellent nutrient in roller tube cultures, in which fragments of monkey testis were employed.[6] When virus of all three types was added to

these cultures, cytopathogenic (degenerative) changes rapidly occurred in the outgrowth of fibroblasts. Application to the field of vaccination was evident, because it was found that such tissue culture fluids when combined with oily "adjuvant", on inoculation in monkeys, promptly stimulated high levels of neutralizing antibody.

In 1953, Franklin *et al.* drew attention to the value of a tissue culture system consisting of minced human embryonic kidney suspended in Medium 199.[7] In these cultures, virus was liberated into the nutrient fluid continuously for almost three months. This observation led the authors to remark that "the stage has now been reached where experiments can be initiated to determine whether poliomyelitis virus will grow in containers considerably larger than those previously used."

In point of fact, several attempts to grow virus in larger containers than the usual small Erlenmeyer flasks were successful. In particular, both Kolle flasks and larger roller tubes holding about 20 ml. were used. In a companion paper, Duncan *et al.* reported that several tissues of rhesus monkeys also supported virus growth; cultures of monkey kidney and testis were especially suitable.[8]

All of the above results were reported, before publication, to Dr. Harry M. Weaver, then Director of Research of the National Foundation. In 1952 our group was urgently requested to undertake the production of poliomyelitis virus on a commercial scale. About this time, Dr. Jonas Salk had published a short report that poliomyelitis viruses grown in tissue culture were antigenic in monkeys and that formalin-treated culture fluids were likewise capable of stimulating antibody.[9] In 1953, Dr. Salk reported a preliminary series of inoculations of humans with a vaccine prepared from tissue culture fluids.[10, 11] Medium 199 was employed in the preparation of the virus incorporated in these vaccines.

From early January 1953, experiments on the large-scale production of virus were carried out in the Spadina Division of the Connaught Laboratories, in laboratories quickly and adequately prepared for the purpose. Trial was first made of large "Winchester-type" bottles with fragments of tissue embedded in a plasma clot; the bottles were rotated slowly. Virus yield was satisfactory, but the labour of preparation was too great. The main problem was to find a container which would hold a large volume of fluid, yet in a thin layer, so as not to "drown" the metabolizing tissue. On the recommendation of Dr. L. Farrell, Povitsky or diphtheria toxoid bottles were also tried, and were found very suitable; these bottles hold 500-750 ml. of fluid when placed on their side. The tissue culture system found most suitable was minced monkey kidney in Medium 199. The method finally developed for the growth of all three types of virus in Povitsky bottles was published in 1953 by Farrell *et al.*,[12] and was presented at the Sixth International Congress of Microbiology in Rome in September 1953.[13]

In July 1953, the author assumed his present position, and the active direction of the program passed to Dr. R. D. Defries, Director of the Connaught Medical Research Laboratories. Under his direction, over 3,000 litres of virus were prepared and shipped to the laboratories of Eli Lilly and of Parke Davis in the U.S.A. for conversion into the "Salk vaccine" used in the 1954 field trials.[1, 2, 14]

The research program in Toronto, 1950-1953, was concerned therefore only with a means of mass-producing poliomyelitis-infected tissue culture fluids, and was carried out at the specific request of the National Foundation for Infantile Paralysis. In many ways, our part in the vaccination program was relatively simple. Subsequent developments have indicated that much the more difficult task is to inactivate the virus in the culture fluids with formaldehyde in such a way that the product consistently passes the prescribed tests for freedom from infectious particles. Two other aspects of the current vaccine urgently require modification. Thus, it is desirable to obviate the need for the use of fresh monkey tissue by growing the virus in a strain of cell that can be cultivated in the laboratory for a prolonged period. Furthermore, at least one of the strains of virus now incorporated in the vaccine has biological properties undesirable for inoculation in man.

It will be realized, therefore, that the work recorded here probably represents only the opening phase in the development of a practical poliomyelitis vaccine, and much research lies ahead, particularly in regard to the perfection of a method which will consistently and completely inactivate the virus without destroying antigenicity.

REFERENCES

1. FRANCIS, T. JR. *et al.*: *Am. J. Pub. Health* Suppl., **45**: 1, 1955.
2. VAN RIPER, H. E.: *Canad. J. Pub. Health*, **46**: 425, 1955.
3. THICKE, J. C. *et al.*: *Canad. J. M. Sc.*, **30**: 231, 1952.
4. MORGAN, J. F., MORTON, H. J. AND PARKER, R. C.: *Proc. Soc. Exper. Biol. & Med.*, **73**: 1, 1950.
5. FRANKLIN, A. E. *et al.*: *Ibid.*, **79**: 715, 1952.
6. WOOD, W. *et al.*: *Ibid.*, **81**: 434, 1952.
7. FRANKLIN, A. E. *et al.*: *Canad. J. M. Sc.*, **31**: 64, 1953.
8. DUNCAN, D. *et al.*: *Ibid.*, **31**: 75, 1953.
9. SALK, J. E. *et al*: *Fed. Proc.*, **11**: 480, 1952.
10. SALK, J. E.: *J. A. M. A.*, **151**: 1081, 1953.
11. *Idem*: *Pediatrics*, **12**: 471, 1953.
12. FARRELL, L. N. *et al.*: *Canad. J. Pub. Health*, **44**: 273, 1953.
13. RHODES, A. J.: Proceedings of Sixth International Congress of Microbiology, Rome, **3**: Sez. viii, 171, 1953.
14. FARRELL, L. N. *et al.*: *Canad. J. Pub. Health*, **46**: 265, 1955.