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VALUE OF NEWER TISSUE CULTURE METHODS IN EPIDEMIOLOGICAL INQUIRIES: AN ILLUSTRATIVE OUTBREAK OF POLIOMYELITIS*

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THE STUDY OF POLIOMYELITIS has been enormously facilitated by the discovery of Enders, Weller and Robbins[9] that the virus will grow in tissue cultures. This technical advance has been widely used for virus isolation and diagnosis, serological investigation, and vaccine production.[8, 19, 22] As yet, however, relatively few outbreaks have been investigated epidemiologically by means of these techniques.[2-4, 11, 12, 23]

Prior to the introduction of tissue cultures, the only method of isolating poliomyelitis virus was the time-consuming and expensive one of inoculating monkeys. Consequently, investigations into the manner of spread of infection depended largely on the validity of the clinical diagnosis of poliomyelitis. The clinical diagnosis of the ambulant and non-paralytic form is, however, notoriously inaccurate. Undoubtedly, in the past, Coxsackie and other infections have been erroneously ascribed to poliomyelitis.[5, 18] In earlier studies, investigators have rarely had the opportunity to assign the poliomyelitis strains isolated to one of the three standard types. This was a serious drawback, and in a study reported by our group some years ago, a child (R.A.) was incriminated as the probable introducer of infection into a small Ontario country town, on the basis of epidemiological evidence supported by virus isolations.[21] Typing of the strains then isolated by the new methods has shown that R.A. was, in fact, infected with Type 2 virus, whereas the other victims were all infected with Type 1. Clearly, R.A. was wrongly blamed and the real source of the outbreak was not traced.

Tissue cultures, especially trypsinized monkey-kidney cultures, afford the means of overcoming most of the difficulties encountered in earlier studies. Culture tubes can be prepared readily and in quantity, and are very sensitive to poliomyelitis, as well as Coxsackie B and "orphan" viruses. Since, in Canada, Type 1 strains cause about 90% of clinical poliomyelitis, finer serological methods of characterizing the antigenic structure of particular epidemic strains would be of great value in tracing the spread of infection.[7] Such methods are not yet available.

The present paper describes a poliomyelitis outbreak which occurred in April 1955 in Toronto. The new tissue-culture methods were used as an adjunct to the conventional methods of epidemiological inquiry. The outbreak is of interest because it appeared to be initiated by an infected resident recently returned from Mexico. Examples of such introduction of infection have more commonly been described in remote communities.[15, 16] It is also of interest that although the strain was highly communicable and invasive, the outbreak terminated within the space of only a few transfers.

METHODS

Trypsinized monkey-kidney monolayer cultures were prepared by Melnick's modification[13] of the methods introduced by Dulbecco and Vogt[6] and Youngner.[24] Epithelial sheets were grown in Synthetic Medium No. 199 containing 1-2% horse serum;[14] at the time of infection, the nutrient was changed to Medium 199 (without serum). Penicillin (500 units/ml.) and streptomycin (250 micrograms/ml.) were added to all media.

ISOLATION OF VIRUS

A suspension of fæces (10-20%) was made in Hanks' balanced salt solution by shaking with glass beads. The

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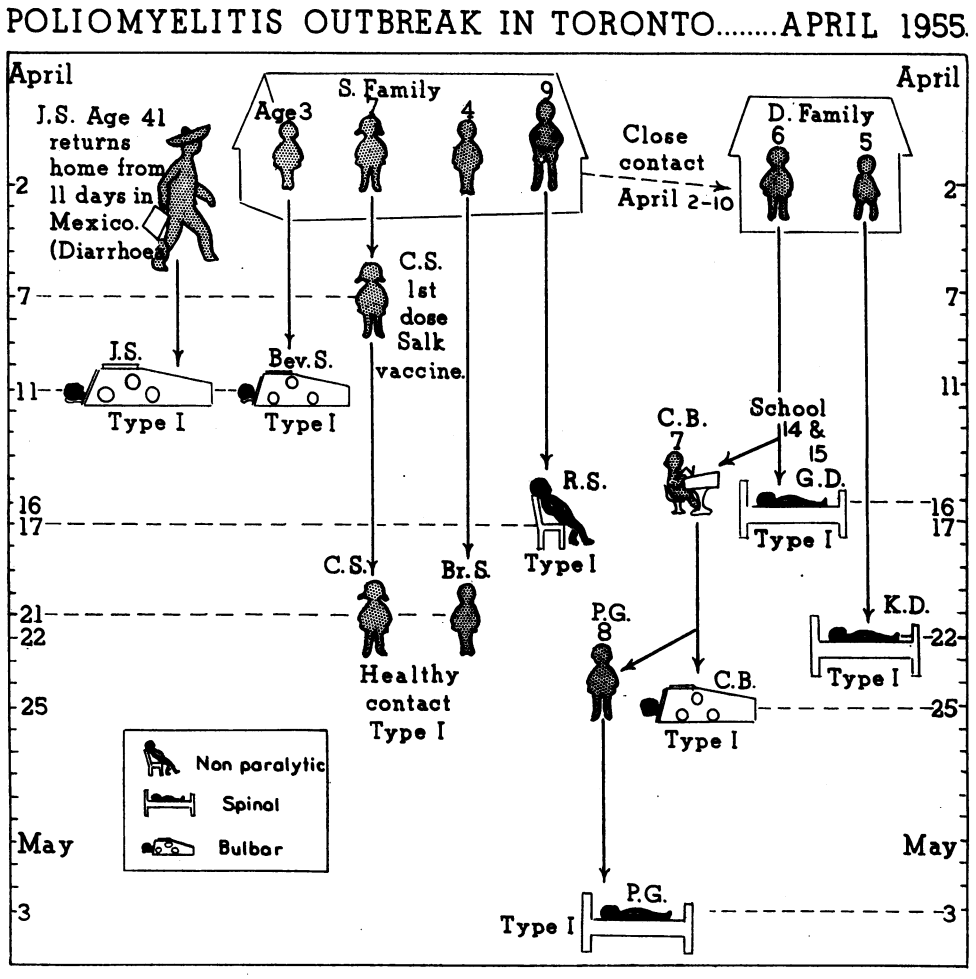
suspension was centrifuged in a Spinco ultracentrifuge at 15,000 r.p.m. for 30 minutes; the supernatant fluid, after the addition of penicillin (500 units/ml.) and streptomycin (250 micrograms/ml.), was inoculated into well-grown monkey-kidney monolayer tissue cultures. Rectal swabs were placed in 1 ml. of Hanks' balanced salt solution containing antibiotics, and these suspensions were used to inoculate tissue cultures.

Cultures were examined daily under the low power of the microscope for cytopathogenic changes. Cultures in which the cells showed no such changes were kept for one week, and were then subcultured into another series. Cultures showing cytopathogenic changes were subjected to examination by "typing";[7] tissue-culture fluids suspected to contain virus were mixed with equal volumes of hyperimmune monkey antisera prepared for the three serological types of poliomyelitis virus. After the mixtures had stood for one hour at room temperature, groups of cultures were inoculated. It was often possible to read the preliminary results after 24-48 hours, but the cultures were left for one week before the final readings were made.

SEROLOGICAL TESTS

Neutralizing antibody titrations were performed by the pH colour test introduced by Salk *et al.*[20] Serial twofold dilutions of serum were made in 0.25 ml. volumes, and an equal volume of 1:100 suspension of trypsinized monkey-kidney cells was added. One hundred CPD_{50} of strains of each type of poliomyelitis virus in 0.25 ml. were then added, and the tubes were sealed with a layer of sterile mineral oil.[25] The results were read after 10 days, by observing pH changes and examining microscopically for the presence or absence of cells. Each test included, in addition to a serum control, a cell, virus, and gamma globulin titration, to check the reproducibility and sensitivity of the tests. Acute and convalescent phase sera from each patient were tested at the same time. The antibody titre was expressed as the initial dilution of serum in the last tube showing complete protection of the cells by the serum against the cytopathogenic effect of the virus.



DESCRIPTION OF THE OUTBREAK

The sequence of events is illustrated in the figure, and the clinical features are outlined in Table I. J.S., aged 41, returned from a trip to Mexico on April 2, 1955. He was feeling unwell and had mild diarrhœa, but went to work on April 4. He fell ill again on April 11 with fever, drowsiness, headache, and vomiting. Difficulty in swallowing developed the next day, and on April 13 he was admitted to hospital. Later, he developed weakness of his shoulders. His youngest daughter Bev.S., aged 3 years, also fell ill

on April 11 with similar symptoms and was admitted to hospital on April 13. She later developed a lower motor neurone facial palsy.

The next patient was R.S., aged 9, who developed non-paralytic poliomyelitis on April 17. The other children in the "S" family, C.S. aged 7, and Br.S. aged 4, remained healthy. Rectal swabs were taken on April 21. C.S. had received her first dose of poliomyelitis vaccine ("Salk type") on April 7.

TABLE I.

POLIOMYELITIS OUTBREAK IN TORONTO, 1955: CLINICAL FEATURES

	Date of onset				
Initials	*Minor illness*	*Major illness*	*Quarantine imposed on family*	*C.S.F. findings*	*Type of disease*
J.S.	2/4/55	11/4/55	14/4	350 cells, mainly lymphocytes	Bulbospinal
Bev.S.	None	11/4		108 cells, all lymphocytes	Bulbar
R.S.	None	17/4		51 cells, mainly lymphocytes	Non-paralytic
G.D.	16/4	19/4	19/4	300 cells, mainly polymorphs	Spinal
K.D.	None	22/4		34 cells, 80% lymphocytes	Spinal
C.B.	None	25/4	28/4	90 cells, 80% lymphocytes	Bulbospinal
P.G.	26/4	2/5	3/5	300 cells, 90% lymphocytes	Spinal

The "D" family lived opposite, and their two children at home, G.D. (aged 6) and K.D. (aged 5), were constantly in the S. garden playing and having meals during the Easter holiday period (April 2 to 10). On April 14, G.D. returned to the private school where she was a day pupil. This school draws pupils from a wide area of Toronto. On April 16, she was feverish, but recovered in the next two days, though she did not return to school. On April 19 she was admitted to hospital with preparalytic poliomyelitis; there were 300 cells per c.mm. in the C.S.F. Later, left triceps weakness developed. On April 22, K.D. fell ill with a similar illness; there were 34 cells per c.mm. in the C.S.F. Weakness of the intercostals developed later.

C.B. (aged 7), a classmate of G.D., who had only been in contact with her on April 14 and 15, and lived some miles away, fell ill on April 25 with bulbar poliomyelitis. The last case in the outbreak was P.G., aged 8, who was a neighbour and friend of C.B., but did not attend the same school. She fell ill with mild paralytic poliomyelitis on May 3. This child had been given gamma globulin on April 30.

Routine quarantine measures were taken against the families of the cases of poliomyelitis as soon as the case was reported to the health authorities. The dates are shown in Table I. These measures consisted in "placarding" and confining all members of the family to the house for seven days from the time of their last contact with the clinical case. The development of poliomyelitis in C.B., who had been in contact with G.D. at school, raised a serious problem. The children in the class were excluded from school and many received gamma globulin on the recommendation of their pædiatricians. No more cases occurred in the school, and only one more clinical case was reported in the whole of Toronto during the ensuing month. This patient had no apparent connection with the outbreak now described and lived some miles away.

VIRUS STUDIES ON PATIENTS AND CONTACTS

Stools were obtained from all the seven patients, and all yielded Type 1 poliomyelitis virus, as shown in Table II. In addition, Type 1 poliomyelitis virus was isolated from the two healthy "S" children, Br.S. aged 4 and C.S. aged 7.

Table II also shows the serological results. It will be seen that four patients (J.S., R.S., C.B., and P.G.) had fourfold or greater rises in antibody to the type of virus isolated from their stools. Two cases (Bev.S. and G.D.) had equally high antibody levels to the infecting type of virus in acute and convalescent sera. One of these cases (C.B.) showed a rise in Type 2 antibodies, and another (P.G.) a rise to Type 3. Only one sample was taken from the remaining individuals and all showed a high antibody level to the infecting Type 1 strain. It is interesting that both adults had titres of over 1:16 against all 3 types of virus, whereas the children, although they had high titres to the causal Type 1 strain, had only low titres against Types 2 or 3.

TABLE II.

POLIOMYELITIS OUTBREAK IN TORONTO, 1955:
VIRUS ISOLATIONS AND POLIOMYELITIS NEUTRALIZING ANTIBODY LEVELS

Initials	Age years	Type of disease	Serum collection (days after onset) S1*	S2*	Neutralizing antibody titre to following** types of poliomyelitis virus 1 S1	1 S2	2 S1	2 S2	3 S1	3 S2	Poliomyelitis virus isolated from stool
J.S.	41	Paralytic	4	23	64	1024	16	16	64	64	Type 1
Bev.S.	3	Paralytic	2	20	256	256	>2	>2	>2	>2	Type 1
R.S.	9	Non-paralytic	3	17	16	64	>4	>4	>4	>4	Type 1
G.D.	6	Paralytic	1	35	256	256	>4	>4	>4	>4	Type 1
K.D.	5	Paralytic	6	33	—	256	—	>2	—	>2	Type 1
C.B.	7	Paralytic	4	4 mos.	128	4096	>2	32	>2	>2	Type 1
P.G.	8	Paralytic	3	4 mos.	512	8192	8	8	>2	8	Type 1
C.S.	7	Healthy		19	—	256	—	4	—	>4	Type 1
Br.S.	4	Healthy		19	—	256	—	4	—	8	Type 1
Mrs.S.	—	Healthy		19	—	256	—	64	—	64	Not tested

*S1 = Acute and S2 = convalescent sample.
**Initial serum dilution.

DISCUSSION

The usefulness of tissue-culture methods in confirming the clinical diagnosis of poliomyelitis is once again demonstrated. The virus was readily isolated from the stools of all seven patients, in addition to two contacts. The first two cases were not at the outset typical clinically and, in view of the rarity of poliomyelitis in Toronto as early in the year as April, the laboratory confirmation of the diagnosis was valuable. In our experience, with trypsinized monkey-kidney cultures, virus can be isolated from nearly every case of clinically typical paralytic poliomyelitis. Similarly, other workers have found it possible to confirm the diagnosis in the laboratory in about 90% of paralytic cases.[8, 10]

Four of the six patients from whom two phase serum samples were obtained showed a four-fold or greater rise in antibody titre (Table II), which would be sufficient to establish the diagnosis even in the absence of virus isolation. Two patients had as high a titre in the acute as in the convalescent sample, so that the serological results alone did not establish a diagnosis of recent poliomyelitis infection. The occurrence of such antibody patterns illustrates that virus isolation is a more sensitive method of diagnosis, and is of course much faster.

The spread of infection in this outbreak could be postulated by observing clinical cases, and this was amply confirmed by the virus isolation tests. All the six children in the "S" and "D" families became infected with virus, and four suffered from clinical poliomyelitis, illustrating both the communicability and invasiveness of the Type 1 strain involved.

The origin of the outbreak is suggestive but not proven. In fact, since J.S. and Bev.S. became ill on the same day, it might be supposed that they both became infected at the same time, soon after J.S.'s return from Mexico. However, the absence of clinical poliomyelitis in Toronto, before or for a month after this outbreak, makes the alternative hypothesis that the virus was in fact imported from Mexico by J.S. very attractive. If this is correct, J.S. almost immediately on return from Mexico infected Bev.S., who developed symptoms after an incubation of not more than nine days.

The virus spread readily amongst the "S" and "D" children in their homes, yet by contrast appeared unable to spread amongst the school children of similar social background, and presumably therefore of similar susceptibility. In the "S" and "D" families, all the children were infected and 4 out of 6 had clinical manifestations. It is well known that there is a high infection rate amongst the family contacts of cases of poliomyelitis, and that such family contacts show a higher rate of clinically apparent infections than the general population.[3, 4, 12] For similar reasons, multiple clinical cases are common in nursery school outbreaks, whereas they

are rare in day schools of the type involved in this outbreak.[17]

It is often assumed that widespread subclinical infection none the less does take place in day schools, but there is very little direct evidence available. In a school outbreak in New York State recently reported by Winsser,[23] there were nine cases amongst 85 public school children all caused by Type 1 virus. Nine days after the last case, 300 stools or anal swabs were obtained from the school children and from home contacts. Only two poliomyelitis viruses (both Type 1) were isolated from these healthy contacts. The sampling may have been done too late to detect subclinical infection, or alternatively the contact at school may not have been close enough to spread the infection. This latter explanation seems more probable since it is well established that virus may be excreted for four or more weeks in the stool.

In view of the recent recommendation of stricter quarantine measures in the prevention of the spread of poliomyelitis,[1, 11,22] it is of interest that virus was recovered from Br.S. and C.S. just as the quarantine restrictions were lifted on the "S" family. As is customary in North America, the quarantine period was for seven days and applied only to the family associates, and not to close extra-familial contacts.

The dates on which quarantine was imposed on the families are shown in Table I. It is clear (Fig. 1) that the "D" family was already infected at the time the "S" family was quarantined on April 14. G.D. became ill two days later, and the family was put in quarantine on April 19. If both the "S" and "D" families had been placed in quarantine for three weeks on April 13, the day J.S. was admitted to hospital, as recommended by the World Health Organization Expert Committee on Poliomyelitis,[22] it seems probable that both C.B. and therefore P.G. would have escaped infection. This illustrates the need for emergency action on the part of the practising physician and of the health official, since G.D. went back to school on April 14. It is instructive to note that had these stricter quarantine measures been employed, the termination of the outbreak might well have been ascribed thereto, since this was the first outbreak of the year and appeared to have been imported. In fact, only two more cases occurred even without any tightening of the usual public health measures.

SUMMARY

1. An outbreak of poliomyelitis in Toronto in April 1955 is described.

2. The value of tissue-culture methods in the epidemiological investigation is emphasized.

3. The outbreak appeared to be introduced by a person infected in Mexico.

4. The Type 1 virus, which was both communicable and invasive, was recovered from all the seven patients and two family contacts.

5. One child attended a day school in the minor illness phase of infection, but only one secondary case developed in the class.

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RÉSUMÉ

Les auteurs désirent mettre en évidence le progrès apporté en épidèmiologie par la découverte de Enders, Weller et Robbins de la conservation en tissu de culture des virus de la poliomyélite. Les considérations présentes s'appliquent à l'épidémie qui a sévi à Toronto en avril 1955 et dont la source aurait été un résident de la localité récemment revenu du Mexique.

Le milieu de culture employé dans ces recherches provenait du rein de singe traité à la trypsine. Les virus étaient isolés des matières fécales. Lorsque, à l'examen microscopique quotidien, des altérations cytopathogéniques étaient notées, les tissus étaient soumis au typage grâce à un anti-serum de singe préparé d'après les trois types sérologiques de virus de poliomyélite. Le titrage des anticorps neutralisants était pratiqué d'après la méthode de Salk. Suit une brève description de l'épidémie et de ses sept principaux cas. Le virus incriminé aurait été du type no 1, d'ailleurs responsable de 90% de tous les cas vus au Canada. Il est à remarquer que les 2 malades adultes possédaient un titre de 1:16 contre les trois types de virus, alors que les enfants n'avaient un titre élevé que pour le type no 1. La valeur de la méthode nous est montrée par la confirmation qu'elle apporte au diagnostic clinique de poliomyélite difficile à soutenir au Canada en avril. Deux malades montrèrent un titre sérologique aussi élevé pendant la phase aiguë de la maladie qu'au cours de leur convalescence, ce qui fait ressortir l'avantage de la méthode basée sur l'isolation du virus en comparaison de celle bassée sur la variation des titres d'anticorps. On doit remarquer la rapidité avec laquelle le virus se propagea chez les enfants de deux familles, en contraste avec la contagion très limitée qui eut lieu à l'école. Il semblerait qu'une période de quarantaine de 3 semaines pourrait remplacer avantageusement la période de 7 jours en vigueur dans notre pays. M.R.D.

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WHAT MAY WE EXPECT OF THE AUTOMOBILE SEAT BELT?

TWENTY-TWO MORE CRASHES WITH BELTS

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AT THE OUTSET, let it be clear that no brief is to be made for the seat belt as distinguished from any other restraining device. The seat belt was originally devised to keep airplane pilots in their seats in rough weather, but it has been called upon to do things for which it was not designed. We need a new device, designed to keep the motorcar occupant in his seat.

The important thing for the modern motorist to understand is that his major problem is to avoid blows to his head. Rare is the motorist with an adequate concept of the forces that assail him when his car comes suddenly to a stop. He must come to realize that when the car stops suddenly, he continues forward at the same speed the car had at the moment of impact. As stated by Drs. Marsh and Moore,[5] "The fall out of an airplane will not hurt you, but the sudden stop will."

As pointed out by Harper,[4] when a 200-pound individual comes to a stop from a speed of 30 miles an hour, the force of his impact will vary from one and a half tons up to *eighteen* tons, depending upon whether he comes to a stop in two feet or two inches. Most people can absorb a blow of one and a half tons if the force is fairly well distributed. If the force is distributed evenly over the whole surface of the back, even eighteen tons can be absorbed with survival. But in the usual crash, these gigantic forces are concentrated upon relatively small areas, the face, the head, the chest, with the results that would be expected if the magnitude of these crash forces were understood.

In 1942, Mr. Hugh De Haven[3] published a study of basic significance to this problem. He reported 10 cases of survival in falls from heights up to 150 ft. during which speeds up to more than 60 miles per hour were attained. Survival was achieved: (1) because the force was widely spread over a large area of the body; and (2) because the contacting surfaces allowed from four to 18 inches (average between eight and nine inches) for the absorption of the fall. This brilliant study, and others which have followed it, clearly indicate that planned arrangements can be made whereby the human body can survive falls or crashes which have heretofore been supposed to be necessarily fatal.

The logical way, of course, to prevent traffic injuries is to prevent the "accident", and we must redouble our efforts to do this. The medical profession has yet to comprehend its responsibility in regard to selection and training of drivers. Thousands of persons are driving cars on our streets and roads who should never have been allowed to do this, *and their doctors know it.* Crashes with these people as drivers are no accident, they are inevitable. We must co-operate and even lead in work on the various methods to prevent "accidents."

However, an approach that has been but little explored is the prevention of injury as distinguished from prevention of the accident. It is the considered opinion of this writer that nothing that we can do will produce such a definitive drop in our motorcar death and injury rates as active measures to prevent injuries in spite of accidents.

The magnitude of these crash forces has been indicated, and there are two methods of dealing with them. One is to pad the surfaces that the motorist will strike as he hurtles forward, and the other is to prevent his hurtling forward.

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