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HISTORICAL ASPECTS OF THE DEVELOPMENT OF LIVE VIRUS VACCINE IN POLIOMYELITIS*

BY

HILARY KOPROWSKI, M.D.

Director, Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania, U.S.A.

To-day, when scientific genius has extended man's intellectual control far beyond the horizons of this planet, it somehow does not seem quite apropos to discuss an infection which, so far as we know, affects the population of only one of the planets and causes illness in only a very small fraction of this population. However, the Committee of the Philadelphia College of Physicians, who have greatly honoured me as a recipient of the Alvarenga Prize, suggested that I should consider vaccination against poliomyelitis as a likely topic, and, acquiescing to their demands, I have decided to trace the history of the development of live polio vaccine.

" History " is one word in the lexicon of all languages which is subject to constant misinterpretation. Hegel was probably right when he said that we learn from history that men never learn anything from history. And there is Fabre's aphorism: " History records the names of royal bastards, but cannot tell us the origin of wheat." I do not intend to lecture about " royal bastards," but perhaps one should remark, in passing, that the relation between a virus disease and legitimate royal offspring was of great importance to the history of France. The younger son of the Duc de Bourgogne, grandson and heir to the throne of Louis XIV, had the opportunity to become Louis XV because his governess was able to hide him when he had measles, thus saving him from treatment by the royal physicians, whose ministrations had already dispatched to the other world his mother, father, and older brother, all of whom had suffered from the same malady (*Saint-Simon at Versailles,* 1958).

Though I am not qualified to discuss " the origin of wheat," I shall attempt to trace the origin of live poliomyelitis vaccine. In doing so I shall be obliged occasionally to dispel certain myths and make sure that the legends and parables surrounding these myths are not substituted for history. I shall do my best to do justice to historical facts, though I am afraid there are some who stand ready to reinstate the myths which I shall dethrone. The statement of Ludwick Wittgenstein, the eminent twentieth-century philosopher: " Der Philosoph behandelt eine Frage; wie eine Krankheit " (The philosopher's treatment of a question is like the treatment of an illness), will dictate the method of my lecture.

The Dawn

On March 15, 16, and 17, 1951, the then National Foundation for Infantile Paralysis called a round-table conference on immunization against poliomyelitis to be held in Hershey, Pa. During the meeting I was asked by the chairman of one of the sessions, Dr. Paul, whether I had any data to present at that time. I shall now quote verbatim the first few paragraphs of my reply:

" The data I want to acquaint you with represent a summary of clinical trials based on oral feeding of children with TN strain of polio (living virus). Properties of this strain were summarized yesterday.

" The TN strain was used at two cotton-rat passage levels: 1st to 3rd and 32nd to 35th. If later we have more time I will summarize, on the blackboard, experimental data of intracerebral inoculation of animals with pools used for feeding purposes. Twenty children and two adults were fed the TN virus and nine of these received material stemming from the 32nd and 35th cotton-rat passage level. The LD_{50} titre in mice of the inoculum ranged from $10^{-3.1}$ to 10^{-4}. Material used for feeding consisted of 10 ml. of 20% suspension of cotton-rat brain and cord infected with TN strain. In many instances the infectious material was mixed with milk and 5% glucose or chocolate milk, and sometimes it was given on a spoon.

" Stools were collected from individuals who were fed virus on the 4th–6th day, the 7th–9th day, the 17–19th day, and the 20th–23rd day."

In continuation of the report, emphasis is placed on the fact that all of the 18 non-immune subjects who remained symptom-free developed antibodies against type II virus as a result of the feeding. In the same report I mentioned two type II immune individuals, neither of whom excreted the virus after feeding and whose levels of antibodies remained the same. This was the first public disclosure of oral administration of living attenuated poliomyelitis virus to a human being, an event which actually took place on February 27, 1950, when a six-year-old boy who had no antibodies against type II virus was given—by Dr. Jervis, Mr. Norton, and myself—an emulsion which tasted like cod-liver oil and was in reality a suspension of cotton-rat cord and brain tissue which had been infected with approximately three million mouse lethal doses of the TN strain (named after my associate, Mr. Thomas W. Norton, who participated actively in the work on the attenuation of this virus).

This boy was the first human subject, anywhere in the world, to be fed live attenuated poliovirus for immunization purposes. He was subjected to carefully controlled clinical and laboratory observation, which revealed that intestinal infection with the virus had been established, without any signs or symptoms of illness. Blood drawn 15 days after oral administration of the TN strain revealed the appearance of specific neutralizing

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antibodies, but we waited 44 days before feeding another type II non-immune subject. After the uneventful period of clinical observation and the development of antibodies in the presence of the intestinal carrier state, virus of the same strain was administered orally to 18 children, bringing the total number to 20 (Koprowski, 1952). This represented the first successful trial of immunization of man against poliomyelitis. Because of its nature, knowledge of this trial was at first confined to Dr. Jervis, Mr. Norton, and myself. No one else knew of the study during the period between February 27, 1950, when the first child was fed the virus and late January, 1951, when observation of the first series of 20 subjects was nearing completion and preliminary evaluation of the procedure indicated that it was harmless.

The Ceryneian Hind

To compare the attenuation of a virus with Heracles' task of taming the Ceryneian Hind is to indulge in what might seem to be exaggeration, but the two tasks are both arduous and time-consuming. Attenuation of poliovirus can most easily be defined as loss of pathogenicity for man, but retention of all antigenic qualities for immunization purposes. Our understanding of the phenomenon of attenuation is very scanty because it has to be judged in relation to virulence, and virulence is one of the scientific terms most easily escaping correct interpretation, particularly in the field of polio research. When the work on polio began in our laboratories in 1947, the criterion for virulence of a poliovirus was its ability to cause paralysis upon direct inoculation into the nervous system of a primate. This criterion is still used to-day, and yet it is, and will, remain a highly artificial one until we can explain the factors determining the presence or absence of paralysis in cases of polio infection in man.

It should be remembered that there is no direct evidence that polio strains isolated from human paralytic cases are all virulent for the central nervous system of monkeys. Conversely, there is evidence for a marked variation in pathogenic properties for monkeys of virus strains isolated from polio-infected but asymptomatic children (Sabin, 1957), but this does not contribute much toward clarification of the problem. Furthermore, the term "virulence" or "neurovirulence" is often misinterpreted as referring to the character of the virus only, with complete disregard of the host. Yet such factors as age (Horstmann, 1955), peripheral trauma, exercise, and stress (Horstmann, 1950) play an important part in the determination of the virulence of a given strain. It has even been proposed that the host factors may be more decisive in the final outcome of a polio infection than the character of the virus (Shope, 1957).

To return from this digression on the theme of attenuation and virulence to the early history of the development of poliomyelitis vaccine, studies on the TN strain of type II virus were conducted for two to three years prior to feeding the virus to man (Koprowski *et al.*, 1951). Since tissue-culture techniques were not available at that time, the virus was subjected, as mentioned before, to numerous passages in laboratory rodents such as cotton-rats and mice and tested sporadically in monkeys for intracerebral pathogenicity.

The same procedure was used for the attenuation of type I virus, which, in contrast to type II, is responsible for most cases of paralytic poliomyelitis throughout the world. In 1952 a mixture of two type I strains, one the virulent Mahoney virus and the other a relatively mild strain called Sickle virus, was injected into mice, using a technique of intraspinal inoculation newly developed by Li and Schaeffer (1953). After adaptation of either the Sickle or the Mahoney virus, or both, to growth in the central nervous system of the mouse, this strain was serially transferred through 27 passages in mice. In 1953 it was judged sufficiently non-pathogenic for monkeys (Koprowski *et al.*, 1954) to be considered as a "candidate" for oral administration to children. By then, thanks to the work of Enders, Weller, and Robbins (1949), tissue-culture systems became available for the study of virus infections, including polio. This method was used in preparing the SM strain of type I virus for administration to man and for the laboratory tests on material excreted by children who were fed the virus.

In 1953 and early in 1954 Jervis, Norton, and I fed the attenuated type I virus to three children who had no antibodies against type I virus (Koprowski *et al.*, 1954a). Again, this was the first attempt to immunize a child against the dreaded paralysis-inducing type I virus by oral administration of an attenuated strain. Two children received type I virus alone, and one, who was also non-immune to type II virus, received a mixture of two attenuated viruses: TN (type II) and SM (type I). None of the children showed any signs of illness and all developed antibodies against the virus they were fed. The child who received a mixture of the two strains developed antibodies against both viruses. Thus a new principle was established, indicating the possibility of immunization by oral feeding of a combination of two or more strains of polio administered simultaneously (Koprowski *et al.*, 1954a). Investigation of the pathogenicity of the attenuated type I virus after passage through the human intestinal tract was included in this study, and showed that passage through man did not result in exacerbation of pathogenicity as tested in monkeys. This completed the first phase of the study of attenuation in the laboratory of two strains of poliovirus which were used successfully for oral immunization of man. The laboratory part of this study was done at Lederle Laboratories.

Other Methods, Other Workers

I will now summarize work by other investigators, employing sometimes similar and sometimes different methods. Roca-Garcia and his colleagues (1952), who were also working at Lederle Laboratories, introduced the use of the chick embryo as another host for adaptation and possible attenuation of poliovirus. They succeeded in infecting a developing chick embryo with a hamster-adapted (Moyer *et al.*, 1952) strain of type II virus (the MEF_1 strain). After 71 passages in chick embryo this egg-adapted strain showed low paralytogenic properties when injected intracerebrally into monkeys (Roca-Garcia and Jervis, 1955). Several years later (in 1956) it was tested for its immunizing capacity in man; however, its ability to establish intestinal infection was found to be poor and its immunizing properties were not outstanding (Roca-Garcia *et al.*, 1956). Progeny of this strain, isolated from virus plaques on monkey-kidney-tissue-culture system, failed in the hands of Plotkin, Koprowski, and Stokes (1959) to establish alimentary infection and antibody response in three out of four infants fed the virus.

In 1954 Li and Schaeffer published their studies on adaptation of the Mahoney type I strain to laboratory mice. The resulting variant was then passaged through monkey-testis culture and monkey skin (Li *et al.*, 1955), yielding a virus of low paralytogenicity for primates. This was made available to Sabin, under the name of LSc strain, and after further studies of attenuation is used to-day by Sabin as a type I vaccine. In 1954 Sabin *et al.* published their first study dealing with laboratory investigations of variants of poliovirus. In the attempt to attenuate the virus Sabin (1955a) made several passages of polio strains every 24 hours in tissue culture, using massive doses of virus as inoculum. At the end of such a passage cycle one passage was made with a very dilute virus inoculum in order to "catch" particles with low pathogenic properties. In 1955 Sabin published a study of feeding of a small number of human subjects with attenuated strains which were subsequently discarded (Sabin, 1955b).

The Stables of Augeias

The introduction by Dulbecco and Vogt (1954) of tissue-culture techniques, which made possible selection of the progeny of isolated particles of a virus and the application of this technique to polio research, has greatly facilitated investigations on the development of attenuated strains. The stables of viruses created by the methods described above—methods which in the light of the new knowledge were rapidly becoming obsolete—could now be cleansed. Though Heracles was to clean the cattle yards of Augeias in one day, "cleaning" of the stables of attenuated viruses was a more time-consuming, though certainly less arduous, task. Instead of diverting the rivers Alpheus and Peneius to flush out the yards, the "cleansing" was done by plating out viruses on tissue-culture monolayers, discarding specimens more pathogenic for monkeys, and subjecting those less pathogenic to repeated "purification," using the same process over and over again.

Variants of all strains of live virus used to-day for oral immunization of man have been selected by this method. The term "selection" instead of mutation is used purposely. There is no available evidence indicating that the long and arduous process of attenuation as applied up to now is anything else than cleaning of a stable of "white-legged black bulls, red stud bulls, and ferocious silvery-white bulls," paraphrasing again from the labours of Heracles. The more ferocious beasts are left behind, the tame animals are selected for breeding, their progeny again are screened for mild character, bred again, and so continued. Those who know their mythology will remember that in spite of solemn promises Heracles received no award for his labour. Augeias even denied that he and Heracles had struck a bargain. Familiarity with the story of live virus vaccine against polio makes one sometimes wonder if one is dealing with a *historical* myth only.

Como tu mi vuoi

More extensive laboratory studies during the past three years permitted characterization of the attenuated strains of polioviruses according to markers other than pathogenicity for monkeys. The death or survival of a monkey injected intraneurally with poliovirus is not an exact criterion of attenuation for man, and the use of many monkeys is expensive. These "laboratory" markers have been developed by several groups of investigators and relate to the following properties of an attenuated strain as compared with a virulent virus: poor growth in tissue cultures kept acid under low bicarbonate content (Dulbecco, 1954); poor growth in tissue cultures of a stable line of monkey-kidney cells in contrast to excellent growth in fresh explants of monkey kidney (Kanda and Melnick, 1959); and high sensitivity to development under high temperatures (Lwoff and Lwoff, 1959).

Estimation of these characters is not a simple laboratory procedure, since knowledge of the existence of the markers is based on the study of kinetic reactions following observation of growth of viruses in one or another system under different conditions. However, these characters of virus have enabled us to "mark" the attenuated viruses. Another extremely useful tool has been developed recently in connexion with serological differentiation of strains of polio within the same type. Studies of Wenner *et al.* (1956), McBride (1959), Gard (1960) and Wecker (1960) indicate that a given strain of a given type can be "marked" serologically so that it can probably be "spotted" at any given moment in its peregrinations. The existence of these markers may finally lead to achievement of the most important goal in the laboratory study of poliovirus: true mutation through chemical change of the infectious nucleic acid. In contrast to selection, which is a somewhat haphazard and prolonged process of attenuation, mutation may in one or two steps radically change the character of the virus.

"On ne badine pas avec 'l'hôte'"

The study of the virus as a virus and not as an entity eliciting response from its animal host is an art, but an abstract art. Piet Mondrian and Jackson Pollack are great painters, but in their special genre. Introduction of the virus into a living host and the reaction of the latter find no parallel in the test-tube. In the case of poliovirus the only real host is the human subject, since he represents the only true susceptible species and has no counterpart in the animal kingdom. In 1953 Jervis, Norton, and I "communicated" to the National Academy of Sciences (Koprowski *et al.*, 1954) results of a comparative study on oral administration of poliomyelitis virus to man and ape. The results are shown in the accompanying Table which is reproduced

*Results of Oral Administration of TN Strain to Three Species of Primates**

Pool No.	Species	Dose of Virus (ml.)	Total No. Fed	No. Excreting Virus	No. Showing Antibodies	Range of Antibody Titre
16	Man ..	1, 5, or 10	9	7	9	1:20–1:500
21	Chimpanzee	10	2	1	2	1:85–1:95
21	Man	10	2	1	2	1:60–1:700
23	Man	10	5	3	5	1:32–1:2,500
24	Man	5 or 10	8	5	8	1:10–1:600
F_1	Chimpanzee	2×10	3	1	3	1:7 –1:300
31	Man ..	5	54	31	54	1:15–1:500
31	Chimpanzee	10	4	0	4	1:2 –1:35
31	Cynomolgus	3×2	11	0	0	—
31	Cynomolgus	2×6	5	0	0	—
YHP N–63	Cynomolgus	3×2	5	1	2	1:2
Grand total	Man ..	—	78	47	78	—
Grand total	Chimpanzee	—	9	2	9	—
Grand total	Cynomolgus	—	21	1	2	—

* In no instances included in this table were signs of illness noted. (Table reproduced from *Proceedings of the National Academy of Sciences*, **40**, 36, 1954.)

from that paper. Here an attenuated type II TN strain was fed to man, chimpanzees, and cynomolgus monkeys. The response in monkeys was poor, in chimpanzees intermediate, and in man excellent. We ended the paper by saying: "It is also clear that for certain questions the crucial answers may have to be sought in man himself, since data obtained in animals may be inadequate."

The Apples of Hesperides

In 1951 the first phase of the original field trial was completed. Confirmation of the results of the trial and more extensive study of reactions in the human host was the next step, and for this it became urgent to arrange for expansion of research in human subjects. The eleventh labour of Heracles was to be undertaken. A fateful meeting took place in New York in the Barbizon Plaza Hotel on January 19, 1952, between Dr. Smadel, at present Associate Director of the National Institutes of Health, Dr. K. F. Meyer, Director of the Hooper Foundation at the University of California, and myself. I was looking for counsel from Nereus and Prometheus. Dr. Smadel was familiar with our work on the immunization of man with living poliovirus and suggested to Dr. Meyer and myself that we establish a co-operative study. This led to prolonged and fruitful collaboration, when the search for the golden apples of the Hesperides was conducted near the Golden Gate, more precisely, in Jack London's *Valley of the Moon*. Dr. T. L. Nelson, Superintendent of the Sonoma State Hospital, became an active collaborator in the study, which began in 1952 and had as its aim elucidation of the following points in the virus–human host interaction:

1. The smallest dose of the attenuated virus which will still infect the human intestinal tract.
2. The duration of intestinal infection and the extent of spread of the virus from the virus-fed subject to non-vaccinated, non-immune contacts.
3. Existence of interference between two or more strains administered simultaneously.
4. Problems concerning different vehicles for feeding the virus.
5. Serial passages of the attenuated strains in human subjects.
6. Administration of attenuated virus in the presence of passively acquired antibody.
7. Local resistance of the human intestinal tract elicited by exposure to attenuated virus.

Plucking of the golden apples in this eleventh labour of Heracles took several years, but the results (Koprowski *et al.*, 1956) of the investigations were very gratifying. The minimum infective dose of type I virus, the then used SM strain, for the human intestinal tract was the equivalent of 2–20 plaque-forming units of the virus. It should be mentioned in passing that infectivity for the intestinal tract of strains of live poliovirus at present available varies considerably (Koprowski, 1958), and that this property may determine the degree of spread of a given virus: those which infect in a smaller dose spread more easily. The duration of intestinal infection varied from 16 days to 104 days for type I virus. (In another study (Koprowski *et al.*, 1956) the record length was extended to 171 days.) The spread of virus occurred from six children who were fed virus to 5 out of 15 type I non-immune contacts, but not to seven non-immune nurses. Administration of more than one strain simultaneously led occasionally to interference—that is, suppression of infection and of antibody induction by one of the strains (Koprowski, 1955).

Administration of one virus to a subject already excreting the other virus which had been fed some time before was an easy method of overcoming interference. Sabin has also observed interference between three polio types administered simultaneously, and suggested feeding the three types in a certain sequence (Sabin, 1956). In the hands of Plotkin, Koprowski, and Stokes (1959), however, this actual sequence of administration of poliovirus types played no part. The only important factor was the spaced feeding. More recent data (Benyesh-Melnick *et al.*, 1959; Horstmann *et al.*, 1959) obtained from the field indicate that the presence of other enteroviruses may interfere with the seeding of the intestinal tract by attenuated poliovirus, and that in some parts of the world, particularly tropical and subtropical areas, such interference may be a difficult problem to overcome. Conversely, opinions have been expressed, particularly by the Russian scientists (M. P. Chumakov, personal communication), to the effect that re-exposure to the attenuated virus, spreading from members of a "non-vaccinated" community, may vaccinate an individual who failed to become immunized on the first attempt either because he had been fed three polioviruses simultaneously or because an enterovirus was present in his intestinal tract.

Results of studies conducted in California have also indicated that the virus can be administered to human subjects in different forms, and that when it is fed in a capsule it will produce intestinal infection but cannot be recovered from the region of the pharynx. These results were confirmed by a study of Paul *et al.* (1957).

Attempts to limit the period of intestinal infection have failed. We are not familiar with any drug that could prevent further spread of the virus, nor is there any known agent for such limitation other than the properties inherent in the virus itself.

One of the last phases of the study in California was conducted in co-operation with Dr. T. L. Nelson, and consisted of serial passages of type I attenuated strain of poliomyelitis virus in man (Nelson and Koprowski, 1957). In this study a type I non-immune child was fed the vaccine and the virus was isolated from the faecal material. The resulting suspension was tested in monkeys, first by the intracerebral and later, in the trials, by the intraspinal route. If no exacerbation of neurovirulence for monkeys was observed—and this was the case—it was made into a bacterially sterile suspension and administered orally to another type I non-immune child, and so on. The results of intracerebral inoculation of the faecal virus preparation into cynomolgus monkeys failed to show an increased pathogenicity in the course of six serial passages of the virus in man. This was confirmed several years later by Smorodintsev (1959) in Leningrad. It should be noted that the type I virus which we are at present employing in mass vaccination programmes does not originate from that used in the initial vaccinations in 1953–6, but was isolated from a child fed the fourth human passage of the virus (Nelson and Koprowski, 1957). This strain was again subjected to numerous laboratory procedures at the Wistar Institute for selection of the least virulent particles, and represents a different strain from that used originally.

Administration of gamma-globulin to children at the time of virus-feeding had no effect whatsoever on the incidence and duration of intestinal infection or active antibody response (Koprowski *et al.*, 1956). In the California study we have observed for the first time local resistance of the intestinal tract of children to reinfection with the TN type II virus three years after the first feeding (Koprowski *et al.*, 1956). These results have been extended to work done with the type I virus (Koprowski, 1958). Subsequently, Fox *et al.* (1957) and Horstmann *et al.* (1957) have shown the same intestinal resistance to infection upon exposure to an attenuated strain in subjects with naturally acquired antibodies. Sabin (1957) has also observed local intestinal resistance to reinfection with the same strain in a certain proportion of subjects when he fed the attenuated strains.

"The Child is Father to the Man"

"Is he? Then in the name of common sense why do we always treat children on the assumption that the man is father to the child?" (G. B. Shaw, *Prefaces.* Constable, London, 1939).

One of the most important aspects of the study of virus–host relationship is the susceptibility of the host at different ages to a virus infection. In order to elucidate these factors in the field of live attenuated poliovirus, a collaboration was established with Dr. Joseph Stokes, of Philadelphia, during the early phase of the original investigations. Studies conducted in co-operation with Dr. Stokes, and later with my colleagues, Drs. Plotkin and Pagano, concentrated on the problem of vaccination of infants less than 6 months old. Preliminary results indicated that infants less than 6 months old can be successfully immunized against poliomyelitis through feeding (in formula) of living attenuated virus (Koprowski *et al.*, 1956), even in the presence of transplacentally acquired passive antibodies.

Similar studies later conducted elsewhere (Martins Da Silva *et al.*, 1957) indicated the complete harmlessness of such an immunization procedure. Infants 2 months of age or older developed intestinal infection at the same rate as older children or adults, and those less than 2 months old were slightly more resistant to the same dose of virus (Plotkin, Koprowski, and Stokes, 1959). However, when the dose of attenuated virus was increased tenfold or hundredfold over that usually fed to older children, establishment of intestinal carrier state, followed by the development of antibody, took place even in infants who ingested virus within a few hours after birth (Plotkin, Koprowski, and Stokes, 1959). Infants who fail to be immunized after the first feeding of virus can be successfully immunized by the same dose repeated when they are somewhat older, thus excluding the possibility that administration of an infectious agent immediately after birth may lead to tolerance and inability to develop protective antibodies later in life. Following administration of virus the passively acquired transplacental antibodies "blend" into the active antibody, which was found to persist after a single feeding of virus for a period exceeding three years (the longest so far tested) (Plotkin *et al.*, 1959).

There is great attraction in the thought that perhaps the newborn population is the ideal population to receive live virus and that too little emphasis has been placed on the host, who in the end reacts to the virus infection. In summary, it may be pointed out that there are numerous advantages in the immunization of young infants against poliomyelitis with living attenuated virus vaccine:

1. The virus can be administered within hours after birth when the newborn are in the hospital, or, if at home, are still under the immediate care of a physician or a midwife. This may assure a more proper administration of the vaccine.

2. Administration of the virus at a time when relatively high levels of transplacental antibodies are present in the serum of the recipient allows "blending" of these antibodies into the virus-induced active type of antibody.

3. Either because of shorter duration of intestinal infection, or because of factors not as yet clearly known, the spread of virus from the vaccinated infant less than 6 months old to his surroundings does not occur very often.

4. In countries where visits to paediatricians or clinics at definite intervals after birth are routine, administration of the two other types of virus may coincide with such visits, thus assuring a proper vaccination procedure. It should be emphasized that in view of the preponderant role of type I virus as the cause of paralytic poliomyelitis, it is our opinion that attenuated type I virus should be administered first. In this way, protection against the greatest menace is guaranteed, even if for some reason the child is never fed the other two types of virus.

5. Immunity after feeding virus to a newborn infant is long-lasting. It is possible that it may last for years, perhaps even a lifetime. There are, of course, no objections to re-feeding the virus at certain intervals during the life of an individual who was vaccinated as a newborn. However, so far there are no indications that this is necessary.

6. Last, lack of opportunity for enteroviruses other than poliovirus to infect the intestinal tract of a newborn within hours after birth eliminates interference and assures successful outcome of the vaccination procedure.

"From Here to Eternity"

Immunity after the administration of living virus may well last "from here to eternity" or "from cradle to grave." Children fed the old TN virus in 1950 (Koprowski *et al.*, 1952) have antibodies against type II virus eight to nine years later, the longest period so far tested (Plotkin *et al.*, 1959). Type I poliomyelitis antibody was present in seven children who were fed the SM strain four years ago, including one who was fed the mixture of type I and II and is still immune to both types. As mentioned before, virus-fed infants retained immunity for a period of two years, and will probably be immune long thereafter. Because of the "head start" of our group in this study, others have not as yet had an opportunity to investigate duration of immunity in subjects they had immunized. However, in a recent communication Sabin (1959) describes persistence of homotypic antibody in five children and six adults fed all three types two years previously. When re-exposed to a massive dose of virus, marked local intestinal resistance was observed against types I and II virus, but the evidence of reinfection with type III virus was frequent.

Tactical Exercises

Thus we have travelled a long way since the time when the attenuated virus was first used. Then, when the work began, it was difficult to project into the future a present that contained only 20 subjects successfully immunized through feeding of the virus. New faith had to be created, since the existing faith was in the development of inactivated virus vaccine only. It was also not too easy to bring over to our side the indifferent and the undecided, since my associates and I were alone in this field when the work began, and remained so for

several years. Gradually, however, other scientists became aware of this problem and joined us in this field of endeavour, which by then was developing rapidly.

In 1957, at the end of what may be called the first era of the history of live virus vaccine, all available experimental and clinical evidence pointed to the conclusion that live attenuated viruses seemed to be safe for use in man. An attempt to organize a field trial of larger magnitude was clearly indicated, and an opportunity for a real "break-through" came when co-operation was established between the Government of the Belgian Congo and ourselves. During 1957 the number of inhabitants of the Belgian Congo who had been fed live virus was rising rapidly, and the "climax" occurred in the beginning of 1958, when a mass vaccination trial of 215,000 subjects, fed the virus at a rate of 12,000 people a day, was organized by Courtois, Jervis, Flack, and myself in the Ruzizi Valley (Courtois *et al.*, 1958). I have found the shortest and best description of this first large-scale live-polio trial in an article published by Chumakov (1959) in connexion with the polio trials conducted in Russia. He said—and I am quoting, translating from Russian—"We have adopted (for the purpose of the Russian trials) a procedure chosen by Dr. Koprowski, who successfully employed the attenuated type I poliovirus (substrain CHAT of strain SM) in the Belgian Congo in 1958 for oral immunization of more than 320,000 children against poliomyelitis. No complications or cases of poliomyelitis were observed among the vaccinated or among subjects who were in contact with the vaccinated individuals."

The Belgian Congo trials have enlarged considerably, and during 1959 75,000 children aged 0–5 years have been vaccinated in Leopoldville (Plotkin *et al.*, 1960). More vaccination campaigns organized in several provinces of the Belgian Congo are raising the number of vaccinated individuals into millions. After preliminary field investigations (Przesmycki *et al.*, 1959) 10 million children and adolescents in Poland are being vaccinated now. Buser and Schär (1958), in Switzerland, and Gard *et al.* (1959), in Sweden, are carefully enlarging their preliminary trials. Russian scientists (Chumakov *et al.*, 1959) in an energetic campaign have immunized millions of subjects with live virus vaccines. Immunization campaigns have been started in South America and Mexico (various authors, 1959). Thus, from a rather modest beginning the live virus vaccination mushroomed during the last two years into a project of considerable size.

Envoi

The history I have just given of the development of live virus vaccine is my attempt to "tell you the origin of wheat." I should not be surprised, however, if you at some time or other hear a different version, put forth by those scientists who follow Schopenhauer's advice on merit: "There are two ways of behaving in regard to merit: either to have some of one's own, or to refuse any to others."

Now that I have talked at such length in my effort to substitute facts for some of the myths surrounding the origin of attenuated poliovirus vaccine, I realize that the time has come to end this talk on justification of my performance.

In the words of Pascal (1656): "Je n'ai fait celle-ci plus longue que parce que je n'ai pas eu le loisir de la faire plus courte."

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EXCESSIVE PERINATAL MORTALITY IN A SMALL TOWN ASSOCIATED WITH EVIDENCE OF TOXOPLASMOSIS

BY

J. S. ROBERTSON, M.B., D.P.H., D.I.H.

Medical Officer of Health, Glanford Brigg Rural District, and Brigg and Barton-on-Humber Urban Districts

During the years 1956, 1957, and 1958, large and persistent differences were noted between the perinatal mortality rates for three adjacent districts in North Lincolnshire. It was found that these differences were due to variation in the stillbirth rates. The small town of Barton-on-Humber suffered a stillbirth rate of 59.6 for the triennium (see Chart). During the same period the neighbouring town of Brigg enjoyed the exceptionally favourable stillbirth rate of 9.0, while the surrounding rural district experienced a rate of 28.1. It was found that the differences in numbers of stillbirths occurring in these districts (Tables I and II) were statistically significant ($\chi^2 = 14.98$; $P<0.001$). This paper describes the investigations which were made in an endeavour to ascertain the cause of the excess of stillbirths experienced in Barton-on-Humber.

TABLE I.—*Vital Statistics for Area*

	Barton-on-Humber U.D. (Pop. 6,420)			Brigg U.D. (Pop. 4,200)			Glanford Brigg R.D. (Pop. 33,200)		
	L.B.	S.B.	N.D.	L.B.	S.B.	N.D.	L.B.	S.B.	N.D.
1956 ..	101	8	3	68	1	2	493	18	7
1957 ..	104	5	3	85	1	2	535	19	6
1958 ..	95	6	0	68	0	2	563	9	9
	300	19	6	221	2	6	1,591	46	22

L.B. = Live births. S.B. = Stillbirths. N.D. = Neonatal deaths (i.e., deaths of infants during first four weeks of life).

TABLE II.—*Perinatal Mortality and Stillbirth Rates* (1956–8)

	Perinatal Mortality Rate*	Stillbirth Rate
Barton-on-Humber U.D. ..	78·4	59·6
Glanford Brigg R.D.	41·6	28·1
Brigg U.D.	35·9	9·0
England and Wales (1956) ..	39·2	22·9

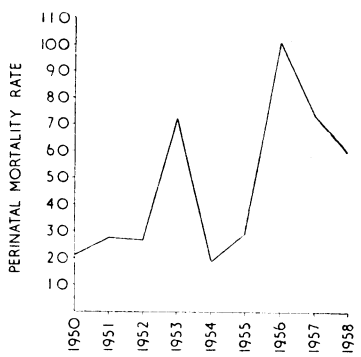
* Note the perinatal mortality rate used in this table is based on stillbirths and deaths below the age of 4 weeks. It differs from the perinatal mortality rate used by the Registrar-General, which only takes account of deaths in the first week of life in addition to stillbirths.

TABLE III.—*Incidence of Gross Malformation and Maceration, Glanford Brigg R.D.* November, 1958–December, 1959 (Preliminary Survey). See also Table VIII.*

	Grossly Malformed	Not Grossly Malformed	Total
Macerated ..	1	5	6
Not macerated ..	5	6	11
Total ..	6	11	17

* Rural district figures included, as only one stillbirth occurred in Barton-on-Humber in 1959.

By the end of 1957 it was already apparent that the excessive mortality in Barton was unlikely to be due to chance, and a preliminary survey was begun. The county medical officer of health kindly arranged for the midwives to complete a questionary in respect of all stillbirths and early neonatal deaths occurring in the three county districts. From these questionaries it soon became apparent that the foetus was either malformed or macerated in almost two-thirds of all stillbirths in the area (Table III). This suggested that the factors responsible must be non-obstetric, and any inquiry must take account of possible genetic and environmental influences if it was to be successful.



Perinatal mortality in Barton-on-Humber, 1950–8.

Environmental Factors

Environmental factors could be chemical, physical, or biological, and the ways in which the two towns might differ in respect of such factors were considered. Both are small market towns within 11 miles (17.7 km.) of each other. Barton is situated on the Humber bank, where the salt marshes and brackish water in old clay pits provide a breeding-ground for mosquitoes, whereas Brigg is situated 10 miles (16 km.) inland on the banks of the River Ancholme—a less favourable breeding ground. Brigg is situated on two main trunk roads and has good rail connexions, whereas Barton is not on any main route. There is a hospital at Brigg, while Barton is remote from hospital facilities. The only other obvious difference between the two towns is in the nature of the local industries. The principal industries in Brigg are fruit-canning, sugar-refining, and hosiery manufacture, while the major industries in Barton are the manufacture of bicycles, chemicals, ropes, and bricks.

Physical or toxic hazards resulting from the nature of local industries might play some part in causing differences in stillbirth rates, and could be investigated by ascertaining the nature of work done during early pregnancy. Remoteness from hospital facilities could