the moisture for these terminal cavities enters by diffusion of air. The amount from each source depends on hydrostatic pressure, on osmosis, and on the activity of secreting cells.

To the water covering the epithelium mucin is added in varying amount, and attention must be directed to this substance. Its presence is of help to ciliary action by giving viscosity to the mucoid blanket, as Proetz has described it; but, though cohesion assists the drag of cilia from a distance, mucin is not essential for ciliary action. Cilia, in fact, work more vigorously in a watery medium, as seen in animalcules such as *Paramecium*. But mucin, a polysaccharide insoluble in water, provides a waterproofing covering to the epithelial surface. This action may be observed in eels, whose thin and flexible skin is covered with viscid mucus. If this slimy covering is removed the fish will, if replaced in fresh or distilled water, take in so much fluid by osmosis as to swell up and die.

The lamellae in the olfactory recess of a fish such as the carp are provided with very many goblet cells, no doubt designed to waterproof the permeable olfactory epithelium (Special Plate Fig. 3).

In the respiratory tract the purpose of a mucoid blanket appears to be similar—to control outward transudation and inward osmosis through the thin and permeable epithelium, while at the same time allowing ciliary action to continue and also permitting passage of olfactory molecules.

In bronchitis excessive collection of fluid in the smaller bronchioles, probably as a result of irritating substances, interferes with respiratory exchanges; in association with this tendency to flooding there is considerable increase of goblet cells, apparently as a well-intentioned effort at protection. But increase of goblet cells entails reduction of ciliated cells, with the result that viscid secretion fills the lumen of the bronchioles and is not removed by ciliary streams.

In asthma a similar process occurs, no doubt dependent in part on increased permeability of cell membranes in an allergic state, with a coincidental rise of pH.

Permeability of cell membranes is influenced by the presence of calcium, a deficiency of which facilitates transudation and osmosis. Administration of calcium gluconate together with dilute hydrochloric acid may be of advantage, especially in cases of nasal allergy, in reducing oedema.

Conclusion

An attempt has been made to show how various evolutionary changes have affected the protection of the respiratory tract of man; these changes, though detrimental to the preservation of health, have been of great advantage in facilitating speech and thereby the development of man's intellect.

A plaque commemorating Dr. Marie Stopes has been unveiled at the entrance to Norbury Park Mansion, Dorking, once her home, on behalf of the Royal Society of Literature. The plaque says: "This house, built for William Lock in 1774, was for many years a favourite resort of Fanny Burney and was from 1934 to 1958 the home of Marie Stopes, scientist and author. By her it was bequeathed to the Royal Society of Literature. The society, of which she was a fellow from 1913 to 1958, has caused this plaque to be erected in her memory and in token of gratitude."

INTERFERENCE PATTERNS ENCOUNTERED WHEN USING ATTENUATED POLIOVIRUS VACCINES

BY

J. H. HALE, O.B.E., M.D., M.R.C.P.

L. H. LEE, M.B., B.S.

AND

P. S. GARDNER,* M.D., Dip.Bact.

*Regional Public Health Laboratory, Newcastle upon Tyne,
and the Department of Bacteriology, University of Malaya*

An account has already been given (Hale *et al.*, 1959; Knowelden *et al.*, 1961) of a trial of Sabin live poliovirus vaccine type 2 in Singapore during the type 1 epidemic of 1958. It was shown that the number of patients who developed paralytic poliomyelitis less than eight days after vaccination was close to that expected from the rates in unvaccinated children, whereas the number of cases which occurred later was lower than the expected number. During the trial we took the opportunity of studying the interference effects between the vaccine strain and the epidemic strain and also between the vaccine strain and other existing enteroviruses.

Recently a further study of Sabin attenuated vaccine types 1, 2, and 3 took place under the auspices of the Medical Research Council. Children from Newcastle upon Tyne co-operated in this study, and we were presented with a further opportunity of observing interference effects.

SINGAPORE TRIAL

The schedules of vaccination and laboratory procedure have previously been described (Hale *et al.*, 1959).

Effect of Type 2 Vaccine Strain on Type 1 Epidemic Strain

Fig. 1 shows the days after vaccination, when clinical cases of poliomyelitis due to the type 1 virus occurred among children who had received the type 2 vaccine. Eight cases occurred within eight days of vaccination, and, as is shown later, these were in children already incubating type 1 infection when given the vaccine. There were two other cases in vaccinated children, but these cannot be included in the analysis, since vaccine was given to one who was actually paralysed and to the other after the onset of illness.

No cases occurred in children between 8 and 19 days after vaccination; it is during this period that multiplication of the vaccine strain in susceptible cells of the alimentary tract takes place and maximum interference effect would be expected. Paralytic cases that occurred later were probably instances of type 1 infection breaking through the heterologous type 2 protection acquired as a result of previous infection induced either naturally or by vaccine.

Effect of Type 1 Epidemic Strain on Type 2 Vaccine Strain

The eight cases of type 1 clinical disease that occurred within eight days of vaccination are presented in Table I.

*Now at the Department of Bacteriology, Royal Victoria Infirmary, Newcastle upon Tyne.

FIG. 1.—Cases of poliomyelitis occurring after type 2 Sabin vaccination. (In Figs. 2-8, ·····=poliovirus 1, —=poliovirus 2, and — · —=poliovirus 3.)

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

From all but one (S.Y.T.), despite repeated attempts, we were unable to isolate the type 2 virus from the faeces. From Case S.Y.T. both types 1 and 2 virus were isolated, but the infection with type 2 was insufficient to produce antibodies, since sera obtained

TABLE I.—*Polio-myelitis Cases Occurring Within Eight Days of Vaccination with Type 2 Sabin Vaccine*

Subject	Sex	Age	Interval between Vaccination and Onset (Days)	Type of Poliovirus Isolated from Faeces
S.Y.T.	M	1 year	2	1 and 2
L.Y.H.	M	2 years	2	1
C.C.	F	10 months	2	1
F.B.S.	F	22 "	3	1
H.B.H.	F	1 year	3	1
W.C.	F	2 years	6	1
K.K.	F	22 months	8	1
W.M.L.	F	5 "	1	Negative

during the acute and convalescent stages of the disease contained antibodies to only the type 1 virus; the titres were 25 and 625 respectively. Case W.M.L. was the only one from which we failed to isolate the type 1 virus. This failure may have been due to the fact that admission to hospital occurred later after onset of illness; serological examinations showed, however, a rise of antibody to type 1 virus from 5 to 56 but none to type 2, indicating that the type 2 virus had never become established.

Effect of Existing Enterovirus Infection on Type 2 Vaccination

Sabin (1959a, 1959b) has reported failure of vaccination due to persistent alimentary infection with non-polio virus strains. Examination of children's faeces immediately before the vaccination campaign in Singapore showed that a proportion were excreting enteroviruses, and a few of these children were followed up after vaccination (Table II). The three children found to be infected with a virus of Coxsackie A type responded in different ways. Two developed antibodies to type 2 poliovirus, although in one the virus was not detected in the faeces; this might have been because faeces were tested on one occasion only. The third child failed to develop type 2 antibodies, despite the fact that the faecal sample examined 15 days after vaccination was found to contain type 2 virus. In this instance, infection with Coxsackie A virus had presumably allowed only minimal growth of type 2 virus, insufficient to give an antibody response. The two children who were found to be excreting monkey-kidney-cell cytopathic agents both developed type 2 antibodies after vaccination, although type 2 was isolated from only one of them. The faeces were tested only once for virus—in this case five days after vaccina-

TABLE II.—*Response to Type 2 Vaccine of Children Excreting Enteroviruses Before Vaccination*

Subject	Sex	Age (Years)	Virus Isolated Immediately before Vaccination	Virus Isolated after Vaccination	Type 2 Antibody Titre	
					Before Vaccination	21 Days after Vaccination
C.T.W.	M	2	Coxsackie A	Poliovirus type 2	<5	<5
P.T.	M	1	"	"	<5	75
L.P.S.	M	2	"	Nil	<5	25
T.A.K.	F	1	Non-polio monkey-kidney cytopathic agent	"	<5	15
S.S.	M	4	" "	Poliovirus type 2	<5	75

tion, which may have been a little early to isolate the type 2 virus. These few cases show that previous infection by an enterovirus does not necessarily result in failure of vaccination.

Effect of Vaccination Campaign on Enterovirus in the Child Population

As non-polio enteroviruses may interfere with establishment of poliovirus infection, so the converse may be expected. Table III shows the results of faecal surveys conducted in Singapore (a) before the vaccination, (b) in vaccinated children four weeks after vaccination, and (c) in non-vaccinated children during the campaign. As expected, the vaccinated group showed a large percentage of children infected with and excreting type 2 poliovirus. Among these children the percentage excreting other non-polio enteric viruses was significantly lower than in the group tested before the

TABLE III.—*Result of Vaccination Campaign on Other Enteroviruses in the Child Population Aged 3 Months to 6 Years*

Group Tested	No. Tested	No. from Whom Virus Isolated		
		Poliovirus Type 1	Poliovirus Type 2	Non-polio Enteric Virus
Children before vaccination	229	7 (3.1%)	0	25 (10.9%)
Vaccinated children within 4 weeks after vaccination	469	0	203 (43.3%)	17 (3.6%)
Non-vaccinated children during vaccination campaign	464	22 (4.7%)	10 (2.1%)	38 (8.2%)

campaign began. Among the non-vaccinated children some were found to be excreting the type 1 epidemic strain and others had apparently become secondarily infected with the type 2 vaccine strain. Although not significant, the percentage excreting non-poliovirus was slightly lower in these children than in those before the vaccination campaign.

NEWCASTLE UPON TYNE TRIAL

Technical Methods

Vaccination Schedules.—Three groups of children aged between 6 and 12 months were vaccinated with the Sabin vaccines according to three schedules. The vaccines were given in a teaspoonful of syrup (simplex B.P.) Group I (13 children) received 10^6 TCD₅₀ of each of the three types simultaneously; group II (10 children) received the same dose, but this was repeated twice more at monthly intervals; and group III (12 children) received 10^6 TCD₅₀ of type 1, followed four weeks later by 10^6 TCD₅₀ of type 3, and finally after a further four weeks 10^6 TCD₅₀ of the type 2 virus.

Isolation of Virus from Faeces

After giving type 1 vaccine, in the case of children assigned to group III, a 10% suspension of faeces was made in 199 medium and spun at 10,000 r.p.m. for 30 minutes in a Spinco centrifuge. Tenfold dilutions of the supernatant fluid were made and 0.1-ml. quantities were inoculated into monkey-kidney culture tubes, two per dilution, which were incubated for seven days. The tubes were examined on the fourth and seventh days for cytopathic effects, which, when they occurred, were shown to be due to type 1 poliovirus by neutralization with type 1 antiserum. From the results of the titration of the supernatant fluid it was possible to calculate the TCD₅₀ of poliovirus excreted per gramme of faeces.

It was not possible to use this method when dealing with faeces from groups I and II or from group III

after the type 3 vaccine had been given, as the children might be excreting two or more different virus types. Neither was it feasible to titrate the viruses in the faeces as before, then by the use of antisera ascertain which viruses were present at each dilution and so assign a titre to each. Experiments showed that in a mixture of viruses the one present in high concentration could prevent the growth of others in low concentration.

Titration of the supernatant fluids in the presence of heterologous antisera also raised difficulties, since the titres obtained were always suspiciously low. Further, it was shown that titration of the vaccine virus in the presence of the two heterologous antisera yielded titres about 4 logs lower. The antisera used were prepared in rabbits by inoculating poliovirus grown in monkey-kidney cultures, and as it seemed unlikely that the sera were non-specific, any effect was probably directly upon the tissue culture cells. Presumably the rabbit antisera also contained antibodies against monkey-kidney cells as a result of inoculation with monkey-kidney-tissue fluids. The sera were therefore adsorbed with cells from monkey-kidney cultures (300×10^6 cells per ml. antiserum) for three hours at 37°C . The vaccine strains were then titrated after neutralization against pools of the two heterologous adsorbed sera. It was then found that the titres obtained were similar to those obtained in the absence of antisera, the inhibitory substances having been adsorbed.

As a result of these findings supernatant fluids from faeces containing a mixture of polioviruses were mixed with equal quantities of pools of (a) types 1 and 2, (b) types 2 and 3, and (c) types 1 and 3 adsorbed antisera at 1/20 and incubated for three hours at 37°C . Tenfold dilutions were made, and 0.1-ml. quantities of each dilution were inoculated into two tubes of monkey-kidney cultures. The amount of virus per gramme of faeces was calculated as before.

The difficulty experienced has been described in some detail as it emphasizes certain dangers in the use of antisera prepared in animals by inoculation of tissue culture fluid from heterologous species.

Interference Between Vaccine Strains

Group I.—Five distinct patterns of response to vaccine were observed in the 13 children in this group. (1) Four children excreted all three types of virus over a prolonged period and produced antibodies to all three types (Fig. 2). (2) One child excreted all three types initially, but types 1 and 3 in low titre and for short duration, and showed only a type 2 antibody response (Fig. 3). (3) Three children excreted types 1 and 2, the latter dominant, but not type 3. Antibodies to types 1 and 2 were produced (Fig. 4). (4) Three children showed an initial excretion of all three types; type 1, however, was of low titre and short duration, so that only types 2 and 3 antibody responses were obtained (Fig. 5). (5) Two children showed an initial excretion of all three types with a short period of type 3 excretion in low titre and antibody responses to only types 1 and 2 (Fig. 6). It was clear that in most cases simultaneous vaccination of the three types resulted in some interference with one or more of the strains. Type 2, however, appeared to be dominant and every child developed antibodies to this type.

Group II.—As was to be expected, the same five patterns were seen after the first dose of the three types of vaccine. However, the second dose, one month after

the first, allowed for colonization of the gut by strains that were interfered with at the first dose, and the example shown in Fig. 7 is typical of this group. One child who excreted all three types after the first dose, and developed antibodies to all three types, gave no response to the second dose, but after the third dose all three types were again excreted, though for a very short duration. In our experience it appears that the second dose will be sufficient to ensure that all three types become established, although a third may act as a booster in some cases.

Group III.—In general, all three types given sequentially produced infection of the gut, and antibody responses to all three types resulted. With three children, despite the absence of antibodies, one of the types failed to colonize. Fig. 8 is a typical example where the type 3 strain failed to become established in a child with no type 3 antibodies. This, no doubt, was due to the type 1 virus being well established and interfering with the establishment of type 3. In the other two cases an established type 3 infection apparently prevented the type 2 strain becoming established. If sequential vaccination becomes the method of choice then the interval between doses should be extended to six to eight weeks to avoid this type of interference.

Effect of Existing Enteroviruses on Vaccination

During the trial four cases of existing enteroviruses interfering with the response to vaccination were observed.

Case 1.—A child in group II was found to be excreting an adenovirus type 1 before vaccination and for seven weeks after. Despite three doses of the mixed types, only poliovirus type 2 was recovered from the stools, and the sole antibody response was to type 2. This was the only child in the group who showed a response to one strain only.

Case 2.—A child in group I responded to the mixed vaccine and excreted all three types of poliovirus for a period of three weeks after vaccination. Cocksackie virus type B5 then appeared in the faeces, poliovirus was no longer isolated, and the Cocksackie excretion went on for a further month.

Case 3.—A child in group II excreted all three types of virus one week after the third dose. Again, Cocksackie B5 virus appeared in the faeces and all poliovirus excretion ceased.

Case 4.—As with Case 3, Cocksackie B5 virus appeared in the faeces after the third dose in a group II child and resulted in the disappearance of the three types of poliovirus present. The excretion of the Cocksackie B5 virus lasted two weeks, after which time polioviruses types 2 and 3, but not type 1, reappeared in the faeces.

In these last three cases the striking finding was the cessation of poliovirus excretion, when Cocksackie B5 virus appeared. This supplanting, however, did not cause any interference with antibody production as it occurred too late, and it probably could not have taken place earlier while the polioviruses were well established.

Discussion

There can be little doubt that enteroviruses exhibit interference patterns in natural infections in very much the same way that interference has been demonstrated in laboratory experiments (Dalldorf *et al.*, 1938; Schlesinger *et al.*, 1943; Domok, 1959). In the Singapore field trial an established infection with Sabin type 2 vaccine strain prevented subsequent infection by the type 1 epidemic strain, and this protection appeared

to be of at least 19 days' duration but probably as long as six weeks. The trial was designed to protect, but also to assess, the safety of the vaccine for both vaccinees and contacts. Since the epidemic was due to type 1 virus it would have been easy to detect cases due to type 2. In the light of our present knowledge of the safety of these vaccination procedures (Smorodintsev *et al.*, 1959; Chumakov *et al.*, 1959; Skovránek *et al.*, 1959) it would be generally agreed that now, faced with a similar incident, the vaccine of choice would be type 1.

Although interference between the enteroviruses was observed, no general pattern was evident. A prior infection might prevent the establishment of a second; the second might be limited in such a way that no detectable antibody resulted; or, further, the second virus might establish itself and apparently obliterate the earlier infection. In all probability the pattern that results is governed by the magnitude of the first infection, the length of time it has been present, and the magnitude of the second infection.

With attenuated poliovirus vaccine large doses such as 10^5 , 10^6 , or even 10^7 TCD₅₀ are used, so that even in communities with a relatively high incidence of enteric virus infection quite good results might be obtained. Not all persons will have infection at the stage capable of interfering, and the dose of vaccine is so artificially large that it might overcome such interference. Also, in a mass campaign a child failing to become infected from the vaccine as a result of interference may become secondarily vaccinated by contact when its pre-existing enteric infection wanes.

The mass immunization with live poliovirus vaccine raises an interesting ecological possibility. It is considered that the live vaccine has a double effect: not only are antibodies produced but the intestinal tract is rendered resistant to subsequent invasion by the same type of virus. Therefore a highly immunized community might contain so few susceptibles that poliovirus circulation would probably cease. These conditions might free the field for infection with other enteric viruses, such as the E.C.H.O. group, resulting in a consequent rising incidence of conditions such as aseptic meningitis. With the disappearance of poliomyelitis, viral meningitis and encephalitis may become a much greater clinical problem.

Summary

Interference between enteric viruses was observed during the mass use of Sabin type 2 vaccine in Singapore in 1958. An established type 2 vaccine infection prevented subsequent infection with the type 1 epidemic strain for at least 19 days and probably longer. In some instances prior infection with the epidemic strain prevented colonization with the vaccine strain.

Interference effects were also observed in the trial in Newcastle upon Tyne. Administration of the three Sabin strains simultaneously as a single dose often resulted in failure of production of antibodies to types 1 or 3 and sometimes to both. Antibodies to type 2 were always produced.

An existing enteric virus infection sometimes prevented the establishment of an infection from the vaccine virus, but whether this occurred depended on the stage of the existing infection. Similarly, poliovirus circulation in a community suppressed the circulation of other enteric viruses.

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TREATMENT OF TETANUS

WITH SPECIAL REFERENCE TO TRACHEOTOMY

BY

P. M. SMYTHE, M.B., M.R.C.P.

AND

A. B. BULL, M.B., F.F.A.R.C.S., D.A.

*From the Departments of Child Health and Anaesthetics,
Red Cross War Memorial Children's Hospital, and
University of Capetown*

In the recent literature on the treatment of tetanus emphasis has been laid on intermittent positive-pressure respiration (I.P.P.R.) as the basic change in technique responsible for therapeutic improvement. But for I.P.P.R. an elaborate organization is required, and in many places neither the equipment nor the trained personnel were available. The value of tracheotomy alone has not been stressed, and the purpose of this paper is to describe the improved results that followed on the introduction of this simpler measure; to define the indications for doing a tracheotomy, or for using I.P.P.R.; and to outline the method of treatment which has been found most effective.

Tetanus is usually classified as severe if the incubation period is less than seven days, or if the period of invasion between the onset of the first symptom and the first generalized spasm is less than 48 hours (Cole, 1940). There are, however, exceptions to this rule, and it has been found more helpful to classify cases according to the severity of the spasms, with special reference to the effect of generalized spasms, laryngospasm, and pharyngospasm on respiratory function. Mild cases have no generalized spasms, only muscular hypertonicity. Moderate cases have generalized spasms, but these are not prolonged or frequent and do not interfere with respiration or swallowing. Severe cases have spasms that impair pulmonary ventilation in one or more of the following ways: prolonged or frequent spasms that fix the chest wall and diaphragm; laryngospasm with complete obstruction of respiration; and pharyngospasm which prevents the swallowing of saliva that lies pooled in the pharynx and is inhaled into the trachea. The impaired pulmonary ventilation is