these poliovirus types. Titrations of the paired sera from children found to have developed antibody showed that six natural infections had occurred—three with type I poliovirus and three with type III poliovirus. In the course of another investigation type I poliovirus had in fact been isolated from two of the children who showed serological evidence of a type I infection.

We conclude that heterotypic poliovirus infections could only have contributed to the late rise in type II antibody in these six instances.

After testing the first post-vaccination blood sample (taken after about two months) we reported that only 77% of 124 children had developed neutralizing antibody following vaccination with TN type II virus (Dane *et al.*, 1957). We were able to obtain blood samples one year later from 15 of the 28 children who did *not* develop antibody. None were found to have developed type II antibody during this interval, thus confirming our original conclusion that the vaccine had failed to "take" in these individuals.

Influence of Heterotypic Antibody on Vaccination with TN Type II Virus

Because of the possibility that previous natural heterotypic poliovirus infections might have influenced the response of children to vaccination with TN type II virus, pre-vaccination samples of blood from 110 children who had been vaccinated with TN type II virus were tested for neutralizing antibody at a 1:4 dilution against types I and III poliovirus. The pre-vaccination antibody patterns found are shown in Table II. Children possessing different pre-vaccination heterotypic antibody patterns showed no significant difference in their response to vaccination.

TABLE II.—*Poliomyelitis Neutralizing Antibody Patterns at the Time of Vaccination in* 110 *Children Fed TN Type II Virus in* 1956

No. of Children	Type I Antibody	Type II Antibody	Type III Antibody
63	—	—	—
20	+	—	—
19	—	—	+
8	+	—	+

—=Titre of < 1 in 4. +=Titre of > 1 in 4.

Discussion and Conclusions

The neutralizing antibody levels which we found in children one year after vaccination with TN type II virus were of the same order as those reported in non-immune children who had received two injections of formalinized vaccine a year before (Report, 1957). A number of the TN-vaccinated children were found to have had a marked decline in their neutralizing antibody level over the year, and our previous finding that 23% of children failed to develop antibody after vaccination was confirmed.

One of the advantages claimed for oral attenuated poliomyelitis vaccines is that they will produce a more durable immunity than formalinized vaccines. We can find no evidence that this was true in a proportion of children vaccinated with TN type II virus. Admittedly our yardstick for the measurement of immunity has been circulating neutralizing antibody. Some sort of local gut immunity follows infection with oral poliovirus vaccines, but in the absence of more precise information about this local immunity it would be unwise to assume that it is more durable than circulating antibody.

Further trials of Koprowski's TN type II vaccine have been abandoned because the virus is liable to change its character and become virulent after growth in the human gut. If it can be shown that the recently developed more highly attenuated poliovirus vaccines do not suffer from this drawback, then it would seem desirable to show also that they can produce a reasonably durable immunity in the majority of vaccinated subjects before they are used on a large scale. On the basis of the results reported here we do not think that durability of immunity should be considered as an automatic benefit associated with living vaccines.

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A SMALL-SCALE TRIAL OF TYPE III ATTENUATED LIVING POLIOVIRUS VACCINE

BY

SUZANNE K. R. CLARKE, M.D.
Lecturer in Bacteriology, University of Bristol; formerly Lecturer in Bacteriology and Virus Diseases, University of Sheffield

A. P. GOFFE, M.B., B.S., Dip.Bact.

C. H. STUART-HARRIS,* M.D., F.R.C.P.
Professor of Medicine, University of Sheffield

AND

E. G. HERZOG, M.B., B.S.
Surgeon Superintendent, King Edward VII Orthopaedic Hospital, Sheffield

From the Virus Research Laboratory, University of Sheffield, and the Wellcome Research Laboratories, Beckenham, Kent

The purpose of this small-scale trial of a single strain of living attenuated poliovirus vaccine was to obtain information concerning the capacity of the virus to induce infection in children previously immunized with inactivated Salk-type vaccine, to measure the antibody formation after the feeding of the virus, and to compare the virulence for monkeys of excreted virus with that of the strain composing the vaccine. In addition, evidence was sought concerning the spread of virus from vaccinated children to their contacts. The virus vaccine selected for use was the type III attenuated poliovirus from culture of monkey-kidney tissue developed by Dr. Albert B. Sabin and obtained as virus-containing culture fluid from Dr. J. D. Verlinde at Leyden. The virus was the highly purified Leon strain previously subjected to many kidney-tissue-culture passages and plaque-selection, and designated the 12a$_1$b (KP4) virus (Sabin, 1957). The strain pool was freshly titrated by one of us (A. P. G.) upon receipt and found to have a titre of $10^{7.35}$ 50% tissue-culture infective doses per ml.

Control Period of Study

The test was carried out in a long-stay orthopaedic institution where the children were under treatment for lesions such as those due to Perthes's disease or tuberculous arthritis. A control period of study began in September, 1957, when immunization with two doses of British-type Salk vaccine was given to certain boys whose parents volunteered for their inoculation. Nineteen boys aged 6 to 15 years received vaccine intramuscularly after a preliminary withdrawal of 10 ml. of blood. The second dose of vaccine was given one month after the first dose, and all inoculations were completed by December 4, 1957. A second specimen of blood was obtained two weeks after the second

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dose of vaccine, and the neutralizing antibody contents of the pre- and post-immunization sera were estimated by titration in monkey-kidney-tissue cultures against each of the three types of poliovirus (Tyrrell *et al.*, 1956).

Permission to use live poliovirus as a boosting vaccine was sought from the parents of those who had received Salk-type vaccine and was given in 14 instances. The children were housed in two wards prior to the trial. One of these contained 12 children aged 6 to 8, of whom five eventually went into the trial, while the remaining nine children in the trial came from a second ward of 12 children aged 9 to 15. Faeces were obtained once weekly for 17 weeks from all children in the first ward and for a further three weeks from all children who were to receive live virus in both wards. In no instance was a strain of poliovirus obtained from the faecal suspensions tested in pools of three to four specimens which were inoculated into monkey-kidney-tissue cultures. This preliminary period of testing was considered desirable because patients convalescent from poliomyelitis were nursed in the same hospital. But their wards were in a separate building situated at least 200 yards (180 m.) from the main block of the hospital. Segregation of the patients in the respective sections was complete. The only natural virus strains recovered in Sheffield at any time from September, 1957, to April, 1958, had been obtained by Dr. J. E. M. Whitehead at the Public Health Laboratory, Sheffield, during the acute phase of illness prior to the transfer of patients with poliomyelitis to the orthopaedic hospital. All were of type I poliovirus. No case of type III poliomyelitis was in fact encountered in Sheffield during the entire period of the trial, and the last case of type III virus isolation had been in December, 1956.

Period of the Trial

The 14 boys aged 6 to 15 years who were to receive live virus vaccine were collected together and were the sole inmates of one ward on February 16, 1958. They were attended by nurses who had been previously immunized with two doses of Salk-type vaccine. All but three of the boys were confined to bed, and some were immobilized on frames during the period of observation.

Virus was fed orally in hard gelatin capsules containing a dilution of stock vaccine virus in sterile synthetic medium 199 mixed with polyethylene glycol 400 so as to contain $10^{4.8}TCD_{50}$ infective doses of virus in a volume of 0.2 ml. A third sample of blood was obtained from each boy prior to virus feeding, which was carried out in two stages.

On February 17 seven boys received capsules containing virus and seven received capsules filled with uninfected culture medium. Faecal specimens were obtained daily or when passed from all the boys during the ensuing weeks, and occasional specimens were obtained from the nurses in contact with the boys.

The second stage of the feeding of virus took place on March 10, 1958, when 11 boys, including all those previously fed dummy capsules, received a capsule containing live virus similar in amount to that previously used. Faecal specimens continued to be obtained at frequent intervals from all children found to be excreting virus, until the specimens were negative. A fourth sample of serum was collected from all the boys on April 1, six weeks after the first virus feeding and three weeks after the second administration of virus. These serum samples, together with the third samples, were titrated for neutralizing antibodies to the three types of poliovirus as before. The results obtained with these sera and also with the faecal specimens are described after details have been given of the technical methods which were used.

Technical Methods: (a) Sheffield

Isolation of Poliovirus from Faeces

Faeces were stored at −25° C. until tested. Emulsions of 10–30% were then made in Hanks's saline, which contained 1,000 units of penicillin and 1,000 units of streptomycin per ml. Each emulsion was centrifuged at 3,000 r.p.m. for one hour, and 0.5 ml. of the supernatant was inoculated into each of four monkey-kidney-tissue culture tubes containing 1.5 ml. of medium. Trypsinized monkey-kidney-tissue cultures were prepared as described by Balducci *et al.* (1956) from rhesus and cynomolgus monkeys. The maintenance medium consisted of 2% calf serum and 0.25% lactalbumin hydrolysate in Hanks's saline to which three times the normal amount of 1.4% $NaHCO_3$ solution had been added. Three to six hours after inoculation the emulsion was removed from the cultures and fresh medium added. When specific cytopathogenic changes appeared the medium and cells were harvested and stored at −25° C. If no cytopathogenic change appeared after 12 days' incubation the cultures were harvested and a second blind pass was made. This was also incubated for 12 days, and if any suspicious cytopathogenic effect appeared a third pass was made. The faeces collected during the control period were tested in pools of three or four specimens, and only one pass in monkey-kidney-tissue culture was made unless suspicious cytopathogenic effects appeared. Throughout the period of the trial, when faeces were collected and examined no live poliovirus of any type was used in the Sheffield laboratory and no faeces from other sources were examined. The neutralization tests on the serum specimens were done before and after this period.

Titration of Faeces

Accurate 10% emulsions were made from most of the virus-containing faeces. Tenfold dilutions of these emulsions were made in Hanks's saline, and 0.2 ml. of each dilution was inoculated into each of two monkey-kidney-tissue culture tubes containing the medium as used for isolation. End-points were calculated by the method of Reed and Muench (1938).

Neutralization Tests

Sera previously stored at −25° C. were inactivated at 56° C. for 30 minutes and serial fourfold dilutions made in Hanks's saline. The same pools of virus were used in each batch of tests. They were diluted in Hanks's saline, so that 50 TCD_{50} were inoculated into each of the tissue-culture tubes. Equal volumes of serum dilution and virus dilution were mixed in screw-capped bottles and allowed to stand at room temperature for one hour. Into each of two monkey-kidney-tissue culture tubes 0.5 ml. of each mixture was then inoculated. The tests were read daily and the end-points calculated for the day after full degeneration had occurred in the control tubes (usually the fifth day after inoculation). The titres were expressed as the reciprocals of the serum dilutions before the addition of virus. The titre was estimated as "2" when the end-point was less than 4 on the final day of reading the test but where some antibody had been detected at readings on earlier days. The titre was expressed as "less than 4" if no neutralization was apparent when a cytopathogenic effect first appeared in the control tubes. However, the technique was not sensitive enough for detection of very small amounts of antibody. The specimens taken before and after Salk vaccination or before and after feeding the live vaccine for each child were tested at the same time. A control serum was included in each batch of tests.

Technical Methods: (b) Beckenham

Virus Titrations

Each virus pool inoculated into monkeys was titrated for tissue-culture infectivity. Trypsinized rhesus or cynomolgus monkey-kidney cultures in roller tubes were used when an abundant healthy monolayer of epithelial cells was present. Cultures were maintained on a protein-free medium devoid of inhibitors which had been shown to be of optimum sensitivity for demonstrating the cytopathic effect of poliovirus; it consisted of 5% horse-liver digest ultrafiltrate in Earle's balanced salt solution (S. E. Smith, personal communication, 1958). Serial tenfold dilutions up to 10^{-9} of each virus were made in this medium, and 0.5 ml. of each dilution was inoculated into 10 cultures each containing

1.5 ml. of freshly changed medium. Cultures were examined up to the seventh day after inoculation, when final readings were taken and the 50% tissue-culture degeneration end-point (TCD_{50}) was calculated by the method of Kaerber (Irwin and Cheeseman, 1939). Each pool selected for virulence study was titrated on the day it was inoculated into monkeys, and again on a subsequent date together with all other virus pools using one tissue-culture batch. Differences between the two titrations varied between zero and 0.5 $\log_{10}$. In Table III the quantities of virus are expressed as TCD_{50} per ml. of culture fluid and are derived from the mean of the titrations.

The vaccine virus has been titrated four times. It was received frozen at −70° C. and was thawed and titrated; smaller lots were distributed into ampoules and refrozen at −70° C. Three ampoules have been thawed for titration subsequently. The first titration gave a value of 7.55 log TCD_{50} per ml. Three titrations of the refrozen material gave values of 6.7, 6.8, and 6.9 log TCD_{50} per ml., indicating a fivefold loss by this procedure. Since twice-thawed material was used for both feeding and virulence tests the value of $10^{6.8}TCD_{50}$ per ml. has been used.

Virulence Tests

A test for virulence, semi-quantitative in nature, was done on the vaccine, as fed to the children, and on two samples of excreted virus from each child proved to be a virus carrier. The first and the last virus strains isolated from the faeces during the study period were tested, except in one child where the virus excreted on the second day after feeding was tested because the first-day specimen contained very little virus. Tissue-culture fluids of the first passage from cultures inoculated with faecal suspensions were pooled when a full cytopathic effect was apparent, and these fluids were used for the virulence experiment.

Each virus pool was inoculated undiluted into two or three monkeys and diluted 1 in 100 into two or three monkeys. Normal cynomolgus monkeys, between 1.2 and 2.9 kg. in weight, received 1 ml. of sample intracerebrally under thialbarbitone, 0.5 ml. being placed in each thalamic region (Bodian, Morgan, and Schwerdt, 1950). Monkeys were observed for up to 30 days after inoculation, during which time they were isolated in groups of two or three in cages measuring 3 ft. (90 cm.) high by 2 ft. (60 cm.) square, and were not removed unless obviously paralysed. It became apparent during the experiment that this method of observation had a high threshold; minor grades of paralysis were almost certainly missed through not exercising monkeys in an open run. Monkeys showing severe paralysis were sacrificed immediately; those showing minor paralysis were watched over for one or two days for further spread; one monkey showing only moderate involvement of both legs on the 14th day was kept until the 29th day with a fair degree of recovery. All surviving monkeys were killed between the 28th and 30th days after inoculation. At post-mortem examination, samples of blood and lumbar enlargement of spinal cord were taken for recovery of virus, after which the whole animal was fixed by perfusion with 10% formol-saline. Selected lengths of lumbar, thoracic, and cervical cord were processed for histological examination.

Results of the Trial

Clinical

No clinical reactions which could be related to the administration of the live vaccine were detected in the boys or in their contacts. Virus excretion which was believed to signify actual infection therefore occurred in the absence of those clinical phenomena, such as pyrexia, minor illness, and so on, which may accompany the non-paralytic form of clinical poliomyelitis.

Virus Excretion

Excretion of the virus was detected in 6 of the 14 subjects who received live virus by mouth, and in five cases was prolonged and quantitatively considerable in amount. Virus strains were recovered from 2 of the first 7 boys fed virus and from 4 of the 11 fed virus on the second occasion. Four who were fed virus on two occasions and who did not excrete virus on the first occasion also failed to excrete virus on the second occasion. Nor were viruses recovered from the seven boys given dummy capsules during the first stage of the trial, when two other boys were actively excreting virus, or from nurses at any phase of the trial. No evidence of spread of virus to contacts was thus obtained, but it happened that all the boys who excreted virus were immobilized on Jones frames during the entire period of the trial.

The amounts of virus detected in the stools of five of the six boys who excreted virus are shown in Table I. The

TABLE I.—*Amount of Virus in Faeces ($Log_{10}TCD_{50}/G.$) from Five Children who Excreted Virus*

Day After Feeding	Case No.				
	1202	1203	1201	1205	1308
1	1·1				1·1
2	4·2	+	−	3·0	
3	4·7				
4			−		4·7
5	4·2	5·0			
6	5·2				3·2
7	5·2		2·2	2·2	
8	4·2	1·7			
9					3·2
10	5·2		+		−
11	3·7				−
12		2·7	+	1·7	
13	3·2	3·2			
14	2·2	2·2			
15	−			1·7	
16					
17	−		2·2		−
18		−			
19					
20	−	−		+	
21			3·0		
22	−				
23			+		
24					
25					
26					
27		−			
28	−				
29					
30					
31		−	+	1·3	
32					
33					
34	−				
35					
36					
37					
38			−	−	

− = No poliovirus isolated.
+ = Poliovirus isolated but insufficient specimen for titration.

sixth boy (1311) excreted a very small amount of virus for only 24 hours after oral feeding. As this virus was detected only after three tissue-culture passes it seems likely that it represented vaccine virus which had passed through but did not multiply in the intestinal tract. The five boys shown in Table I excreted quantities of virus varying from 1.1 to 5.2 $\log_{10}$ 50% tissue-culture infective doses of virus per gramme of stools. Excretion occurred for periods from 9 up to 30 days including the day on which virus was first detected. It is certain that multiplication of virus had taken place in the alimentary tract of these five children. The viruses recovered from each of two specimens of faeces from each child were typed by neutralization techniques. All were found to belong to type III poliovirus.

Antibody Response

The results of serum neutralization tests on all 14 of the boys in whom the trial was made are shown in Table II. The rise in antibodies after the two doses of Salk-type vaccine were of the same order as those found by other workers (M.R.C. Committee, 1957; Russell, 1958). Good antibody responses were obtained in most of the boys, but one boy (1203) failed to respond to the type III antigen and one (1200) to the type I antigen. After the feeding of the live vaccine children who did not excrete virus and the

one boy with transient excretion showed no increase or a twofold decrease in antibody to type III virus and variations with a twofold increase or decrease in antibodies to the other types. Of the five children who excreted virus for prolonged periods one showed no change in antibody levels and one showed a twofold decrease in antibody to type III virus. The remaining three children all showed a fourfold or greater increase in type III antibody without any significant alteration in antibodies to the other poliovirus types. The two children who showed no antibody response after the live virus vaccine had received the second dose of inactivated vaccine 2½ and 4½ months previously. This relatively short interval of time may have been responsible for their failure to develop a further increase in antibody.

In such a small number of subjects it is clearly impossible to discern correlations between the ability of the oral vaccine to establish infection and the antibodies pre-existing in the blood. Of the five who became infected, two had no antibody to type III virus before the administration of Salk-type vaccine, and only one of those who failed to become infected was similarly devoid of such antibody. On the other hand, the geometric mean titre of the type III antibodies just prior to feeding was 42.2 in those who developed infection compared with a mean titre of 351.8 in those who proved resistant to infection. Finally, four of the five boys who became infected were among the youngest subjects in the trial, so that it is possible that age is as important a factor in determining the likelihood of infection as is naturally or artificially acquired antibody.

Neurotropism of Excreted Viruses

The comparative virulence for monkeys of the vaccine virus and of the strains of virus excreted by the five boys considered to have developed an alimentary infection is shown in Table III.

None of the monkeys inoculated with vaccine showed either paralysis or other evidence of neurotropic activity. On the other hand, each child in whom the vaccine virus multiplied excreted at some stage a virus of increased virulence. The viruses included in Table III show a graded spectrum of neurotropic activi[ty] which bore a distinct relationship to the length of t[ime] that the virus had had to multiply after the original feeding. Thus Case 1308 on the first day and Case 1205 on the second day showed viruses of similar behaviour to the vaccine. Cases 1203 and 1202 on the second day showed some slight increase in paralytic activity. Every strain after the second day showed paralytic activity at the lower concentration of virus inoculated. With the exception of Case 1201, in whom the 7th-day and 31st-day virus both showed strains of intermediate virulence, all cases showed an increased virulence in the strain of virus excreted later compared with that excreted in the earlier phase of infection. On the 14th day Case 1203 showed a degree of neurotropism similar to that of field strains of type III virus from paralytic cases. Eight thousand tissue-culture doses given intracerebrally caused severe paralysis of relatively rapid onset in two monkeys and extensive histological lesions in a third monkey. One monkey had viraemia at the onset of paralysis on the sixth day after receiving the lower dilution of virus.

TABLE III.—*Intracerebral Virulence Test in Monkeys*

Case No.	Days after Feeding	Virus Titration*	Estimated TCD_{50} Inoculated	Paralytic Rate	Day of Onset Paralysis	Histological Infection Rate	Virus Recovery from Cord
Vaccine (diluted and refrozen)	—	6·8	6,400,000	0/3	—	0/3	0/3
			64,000	0/3	—	0/3	0/3
1201	7	7·95	89,000,000	1/3	15	3/3	1/3
			890,000	2/3	6, 22	2/3	2/3
1201	31	8·15	140,000,000	2/2	4, 9	2/2	2/2
			1,400,000	1/3	16	3/3	2/3
1202	2	7·65	45,000,000	2/2	9, 9	2/2	2/2
			450,000	0/3	—	2/3	1/3
1202	14	6·85	7,000,000	1/3	8	3/3	1/3
			70,000	1/3	29	3/3	2/3
1203	2	7·1	12,000,000	1/3	19	1/3	1/3
			120,000	0/3	—	0/3	0/3
1203	14	5·9	800,000	3/3	6, 7, 8	3/3	3/3†
			8,000	2/3	8, 10	3/3	2/3
1205	2	6·8	6,400,000	0/2	—	0/2	0/2
			64,000	0/3	—	0/3	0/3
1205	31	6·9	8,000,000	1/3	16	3/3	0/3
			80,000	1/3	28	1/3	1/3
1308	1	7·2	16,000,000	0/3	—	0/3	0/3
			160,000	0/3	—	0/3	0/3
1308	9	6·95	8,900,000	2/2	7, 16	2/2	1/2
			89,000	1/2	15	1/2	0/2

* Mean of two titrations. $Log_{10}TCD_{50}$ ml.
† Monkey paralysed on sixth day also showed viraemia.

As will be noted from Table III, the extent of involvement of the spinal cord revealed by histological examinations was greater than that shown by clinical assessment of the monkeys from outside their cages. The lesions in some cases were indeed extensive and severe, as can be seen from Figs. 1, 2, and 3. All the viruses recovered from the spinal cords of these monkeys were type III. No other strain of poliovirus was being used in the unit housing the monkeys during the period of these experiments.

Discussion

The use of living attenuated viruses for immunization against poliomyelitis is now being actively pursued by several different groups of observers. There are, however, few recorded observations concerning the use of such vaccine as an agent for reinforcing the basic immunity acquired by immunization with Salk vaccine. Paul *et al.* (1957) reviewed the argument for such a use of an attenuated live virus vaccine and suggested that it might be desirable to obtain a planned exposure to an attenuated virus by mouth rather than to rely upon natural infection with an unpredict-

TABLE II.—*Neutralizing Antibody Titres in 14 Children*

Case No.	Age	Type III Virus				Type I Virus				Type II Virus			
		1	2	3	4	1	2	3	4	1	2	3	4
(a) Children who excreted virus after feeding type III virus vaccine													
1201	7	<4	256	128	128	32	512	128	128	512	2,048	2,048	2,048
1202	6	16	64	64	256	128	512	512	512	8	512	512	512
1203	6	4	4	8	512	512	2,048	4,096	4,096	<4	128	64	128
1205	6	<4	32	8	2,048	<4	8	2	<4	2	128	16	32
1308	10	8	256	256	128	32	512	1,024	512	<4	128	128	64
(b) Children who did not excrete virus after feeding vaccine													
*1200	6	32	1,024	512	512	2	2	<4	<4	<16	64	8	16
1203	15	<4	8	16	16	<4	64	64	128	>4	4,096	8,192	4,096
1304	15	32	512	2,048	2,048	8	1,024	512	1,024	<16	256	128	64
*1305	12	16	2,048	2,048	1,024	512	1,024	256	512	128	1,024	256	512
1306	10	4	1,024	4,096	2,048	128	512	256	256	16	1,024	512	256
*1307	14	8	256	512	512	8	64	128	128	128	512	2,048	1,024
*1310	11	2	8	8	8	<16	512	512	512	128	2,048	2,048	2,048
1312	9	128	512	512	256	32	1,024	1,024	512	<4	128	128	128
(c) Transient excretion virus after feeding vaccine													
1311	9	2	2,048	1,024	1,024	<4	8	8	8	8	1,024	2,048	2,048

1=Before Salk-type vaccine. 2=After Salk-type vaccine. 3=Before feeding type III virus vaccine. 4=After feeding type III virus vaccine.
* Fed virus on two occasions.

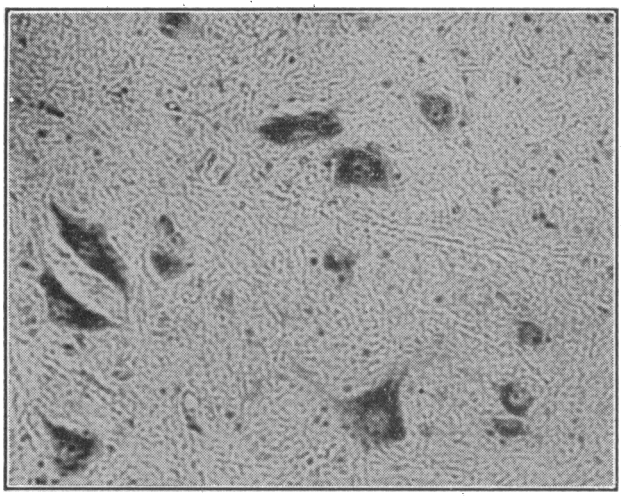

FIG. 1.—Photomicrograph (×170) of lumbar cord of monkey killed 28 days after intracerebral inoculation of $10^{6.8}TCD_{50}$ of vaccine virus. No paralysis, normal histological appearance. Nissl stain.

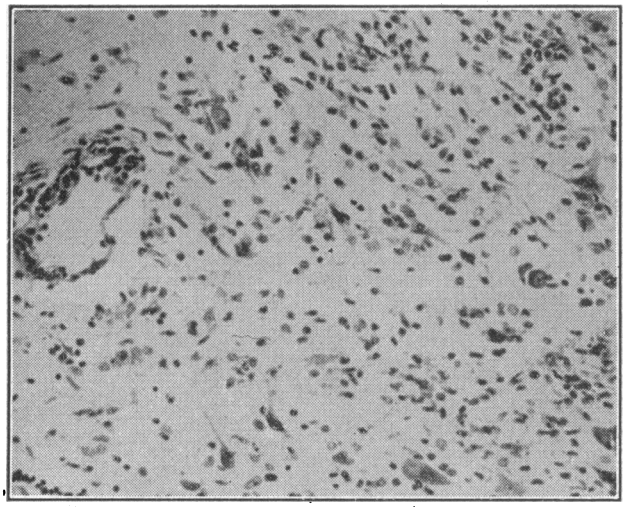

FIG. 2.—Lumbar cord of monkey paralysed eight days after intracerebral inoculation of $10^{5.9}TCD_{50}$ of virus from faeces of Case 1203 on the 14th day after vaccine. Typical lesions of acute poliomyelitis. Type III virus isolated.

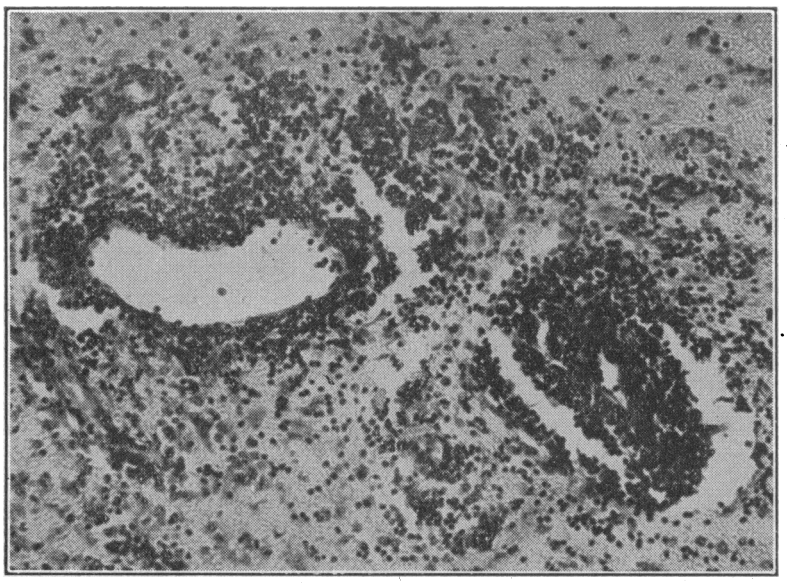

FIG. 3.—Lumbar cord of monkey killed 29 days after intracerebral inoculation of $10^{6.85}TCD_{50}$ of virus from faeces of Case 1202 on the 14th day after vaccine. Extensive lesions typical of convalescent poliomyelitis. Type III virus isolated.

able dose of wild possibly virulent poliovirus. They showed (Horstmann *et al.*, 1957b) that a child of 9 vaccinated previously with Salk vaccine was readily infected by live attenuated type III virus (Leon—KP34) fed by mouth. In a later trial of type I virus (L.Sc. strain) under conditions where spread of infection readily occurred to contacts, Horstmann *et al.* (1957a) found that children immunized with Salk vaccine acquired alimentary infection in 10 out of 10 instances after virus-feeding by mouth and in 12 out of 13 instances by natural exposure. These observations are paralleled by the occurrence of alimentary infection under natural circumstances in previously vaccinated children who were family contacts of cases of poliomyelitis (Lipson *et al.*, 1956; Gelfand *et al.*, 1957; Davis *et al.*, 1958). It thus appears likely that children who have received Salk-type of inactivated poliomyelitis vaccine can readily become infected under natural conditions or after deliberate feeding of poliomyelitis virus. Yet the observations of Sabin (1956) and of Horstmann *et al.* (1957a) showed that persons whose blood contained naturally acquired antibodies frequently resisted infection by live attenuated viruses fed by mouth, thus suggesting that the alimentary tract does acquire immunity after exposure to poliovirus.

Our own study has confirmed the fact that it is possible to infect the alimentary tract of children previously immunized with two doses of Salk-type vaccine in spite of the presence of antibodies in the serum at the time of infection. Too few of the children lacked type III antibody prior to immunization with Salk-type vaccine for us to throw any light on the relative protective power of natural versus artificially acquired antibody, though the data suggested that age of the subject may be a factor in this question of immunity. The circumstances of our trial were also too restrictive for the observation that spread of virus did not take place from carriers to contacts to have much significance. No spread of infection was in fact observed by Horstmann *et al.* (1957b) in the study of a similar attenuated type III virus in a ward of 13 mentally defective children and adults. On the other hand, the L.Sc. type I attenuated virus fed in the later study of Horstmann *et al.* (1957a) to mentally retarded children spread widely, so that strain differences as well as the circumstances of the trial may be important in determining whether or not contact infection will occur.

The antibody-boosting capacity of infection with attenuated poliovirus proved less uniform than was expected. One explanation of the failure of two of the five infected children to react may be that the recent boost of antibody consequent upon the injection of Salk-type vaccine had interfered with the capacity of the alimentary infection to raise the antibodies to a still higher level (Sabin, 1957). However, Dick and Dane (1957), who worked with the rodent-adapted type II strain developed by Koprowski *et al.* (1956), similarly recorded a failure of uniformity in antibody response after oral feeding.

Probably the most important of our observations are those relating to the comparative virulence of the vaccine virus which was fed and that of the strains of virus recovered from the stool. Tests on the vaccine virus fully confirmed Sabin's opinion concerning the high degree of attenuation of this virus for the brain and spinal cord of the monkey. However, all the children whose faeces contained virus for several days after oral feeding excreted strains with a capacity to induce paralysis in monkeys inoculated intracerebrally. Sabin (1957) found that the viruses excreted by persons fed attenuated poliovirus strains had an altered neurotropism in that they would sometimes produce paralysis after direct intraspinal inoculation into monkeys. Some increase in intracerebral activity was also

recorded, more frequently for type III than for types I and II, but less consistently and of a lower order than that recorded above. However, the majority of viruses examined by Sabin were isolated early in excretion; in common with our finding, increased neurotropism was found most often at a later stage of excretion. Nevertheless, Sabin considered that such excreted viruses were less highly neurotropic than wild polioviruses disseminated by healthy children during non-epidemic periods.

It is a matter of opinion whether all the viruses excreted by the children in our trial were less virulent than strains encountered in nature. But the degree of alteration from the original vaccine strain appeared to be considerable. In this regard our observations seem to be similar to those recorded by Dane *et al.* (1957) with rodent-adapted type II virus and by Dick *et al.* (1957) with modified type I virus (Koprowski *et al.*, 1956). It will be recalled that Dick and Dane (1957) stated that these virus strains were unacceptable for mass immunization because of their alteration in virulence during passage through the alimentary tract. In spite of the mass use of living attenuated poliovirus vaccine in the Belgian Congo (Courtois *et al.*, 1958) it is our opinion that further research concerning the properties of the viruses excreted by those receiving such vaccines is desirable in order to reconcile the conflicting evidence.

Summary

Fourteen children who were patients at a long-stay orthopaedic hospital were immunized with two doses of British inactivated poliovirus vaccine and were later given living attenuated type III virus vaccine (Sabin) by mouth in gelatin capsules. No adverse clinical reactions were observed. Five of the children developed an alimentary infection, as shown by excretion of virus in the stools for from 9 to 30 days, and in three instances by a further increase in antibody titre. No instance of spread of excreted virus to contacts was detected. The vaccine virus and some of the viruses excreted early in the course of infection did not produce paralytic effects in monkeys inoculated intracerebrally. All five infected children excreted strains of virus possessing enhanced neurotropic properties for monkeys at a late stage of the infective process.

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COMBINED GASTRIC AND DUODENAL ULCERATION

A SURVEY OF 157 CASES

BY

R. MANGOLD, M.D.

From the Department of Gastro-enterology, Central Middlesex Hospital, London

From the time Sir David Wilkie (1926) first reported on combined ulceration of the stomach and duodenum there has been a considerable divergence of opinion in respect to its incidence. This is revealed in the recent observations by Feldman (1953) and by Johnson (1955). From their papers it would appear that surgeons as a rule observe a much higher proportion than is found in either post-mortem or radiological studies. Johnson also drew attention to gastric retention as leading to gastric lesions in patients suffering from chronic duodenal ulcer. The present study shows the incidence of combined ulcers as encountered in a general hospital, and gives some additional information on duodenopyloric stenosis causing secondary ulcer of the stomach. It also reveals the increased danger of bleeding, making surgical treatment more advisable than with a single ulcer.

Material

The 157 cases reported here were collected from the files of the in-patient and out-patient clinics of the Central Middlesex Hospital, London. Only cases with combined ulcers first diagnosed in the years 1947 to 1954 were included. Definite radiological, surgical, or post-mortem evidence of chronic ulceration was required. Acute ulcers were excluded. In all doubtful cases the *x*-ray films were reviewed. While the work proceeded, it became increasingly obvious that cases with antral, prepyloric, and pyloric ulcers were not suitable for analysis. There would often arise considerable doubt about the exact location of the ulcer. Extensive scarring due to prepyloric lesions will sometimes lead to secondary duodenal deformity, particularly after perforation. For this reason, only gastric ulcers at or above the angulus were included.

Incidence

During the eight-year period from 1947 to 1954, 157 cases showing both gastric and duodenal ulcers or ulcer scars were observed. In 139 of these, both ulcers were diagnosed radiologically. During the same period 3,744 duodenal and 1,352 gastric ulcers were detected by *x*-ray study in the hospital, excluding the combined lesions. Based on *x*-ray findings, the observed incidence is 2.7%. As mentioned above, cases with prepyloric ulcers were not included in the combined ulcer group. Ihre and Müller (1943) observed 26% of all benign gastric ulcers to be prepyloric. Many authors give figures lying between 15 and 20%, but Gott,

TABLE I.—*Incidence of Combined Ulcers Based on X-ray Investigation (Central Middlesex Hospital, 1947–54)*

No. of Cases With Single Ulcers	Combined Ulcers (Prepyloric Ulcers Excluded)		Combined Ulcers (Estimated 25% Prepyloric Ulcers Included)	
	No.	Incidence	No.	Incidence
D.U. .. 3,744	139	$\frac{139\times100}{3,744+139}=3{\cdot}6\%$	185	$\frac{185\times100}{3,744+185}=4{\cdot}7\%$
G.U. .. 1,352*		$\frac{139\times100}{1,352+139}=9{\cdot}3\%$		$\frac{185\times100}{1,352+185}=12{\cdot}0\%$
All ulcers 5,096	139	$\frac{139\times100}{5,096+139}=2{\cdot}7$	185	$\frac{185\times100}{5,096+185}=3{\cdot}5$

* Including prepyloric ulcers.