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Comparison of antibody responses and virus shedding following administration of trivalent oral poliomyelitis vaccines prepared either in monkey kidney or human diploid cell substrates

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SUMMARY

Nineteen (22·9 %) of 83 sera collected before vaccination from adult volunteers aged 21–64 years were without neutralizing antibody to poliomyelitis at levels of 0·15 i.u./ml for types I and II and 0·1 i.u./ml for type III. Some correlations were found between the history of previous vaccination and the presence of antibody but these were not well defined.

Vaccination with a single dose of trivalent oral polio vaccine elicited fourfold or greater antibody responses to one or more poliomyelitis types in 53 (63·9 %) volunteers, the percentage antibody responses being inversely related to the titre of antibody present before vaccination. Types I, II or III poliomyelitis virus were recovered from 76·8 % of faecal samples collected 1 week after vaccination. The percentage recovery progressively declined thereafter until virus was recovered from 10·5 % of samples collected 6 weeks after vaccination.

Type for type, the titres and percentages of antibody responses and virus shedding in faeces were similar following trivalent oral poliomyelitis vaccines whether prepared in monkey kidney or human diploid cell substrates. Some change in reproductive capacity temperature (r.c.t./40) marker was found in faecal isolates from volunteers vaccinated with monkey kidney and human diploid grown vaccines but no change in 'd' marker was found.

INTRODUCTION

In the last 18 months a small but important increase has occurred in the number of cases of paralytic poliomyelitis notified in England and Wales. Twenty cases were notified in the five years 1971–5 and were followed by 10 cases in 1976 and 15 cases in 1977 (Office of Population Censuses and Surveys, 1975, 1978).

Concern about the safety of pertussis-containing vaccines and a decline in their acceptance has led to a fall in the acceptance of simultaneously administered oral

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poliomyelitis vaccine (OPV) (Department of Health and Social Security, 1976). Older persons may never have received either attenuated (OPV) or inactivated (IPV) poliomyelitis vaccines and there are no national recommendations for the administration of vaccines after the school leaving age. Nevertheless, oral poliomyelitis vaccine may transmit from vaccinee to family contacts, and with the occurrence of symptomless natural poliomyelitis the percentage of the population protected against poliomyelitis may be somewhat greater than acceptance rates indicate.

Oral poliomyelitis vaccines used in the United Kingdom are prepared from seed strains derived from those developed by Sabin (Sabin, 1957, 1959). They have usually been produced in substrates of primary monkey kidney cells. However, increasing difficulties in obtaining adequate supplies of monkeys, the risks to manufacturing staff of serious infections in handling them and the presence of numerous simian contaminating viruses leading to a high rejection rate of manufactured batches, point to the use of human diploid cells as an alternative. Human diploid cell strains provide a standardized seed-cell virus substrate and more opportunities for assessments of safety with particular respect to the exclusion of extraneous agents (Hayflick & Moorhead, 1961).

The study reported here was undertaken to survey an adult population for histories of vaccination against poliomyelitis, neutralizing antibodies to poliomyelitis types I, II and III and to compare serological responses and virus shedding following administration of a single dose of trivalent oral poliomyelitis vaccine prepared either in primary monkey kidney or WI38 human diploid cells.

MATERIALS AND METHODS

Eighty-three volunteers (41 females) aged 21–64 (mean age 34·2 years) were recruited from employees of the Wellcome Foundation Limited at four locations in the United Kingdom: Beckenham, Dartford, Berkhamsted and Crewe. In each location volunteers were randomly allocated to vaccination with a single dose of trivalent oral poliomyelitis vaccine prepared in a substrate of either monkey kidney or human diploid cells. The groups of volunteers receiving each vaccine were broadly equivalent in relation to age, sex, history of previous immunization against poliomyelitis and immunization of household contacts with OPV (Table 1). Volunteers were bled immediately before and 6 weeks after vaccination and sera titrated for neutralizing antibodies to poliomyelitis types I, II and III. Faecal samples were collected weekly for 6 weeks after vaccination from 58 volunteers for investigation of virus shedding.

Vaccines

Vaccines compared were a commercially available trivalent OPV prepared in monkey kidney cells, Wellcome lot TRD 234/1 and a trivalent OPV prepared in WI38 human diploid cells, lot PTF 006/1. The human diploid cell vaccine was prepared from working seeds established at the Wellcome Research Laboratories by one further passage in monkey kidney cells of Sabin's original seed virus

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Table 1. *Age, sex and vaccination history of volunteers in comparative study of OPV grown in monkey kidney or human diploid cells*

	Monkey kidney vaccine	Human diploid vaccine
Number of volunteers	38	45
Number female	19 (50·0%)	22 (48·9%)
Age – years (mean ± S.D.)	33·5 ± 10·1	35·0 ± 11·9
Vaccination history		
OPV	14 (36·8%)	6 (13·3%)
IPV	10 (26·3%)	21 (46·7%)
IPV + OPV	7 (18·4%)	5 (11·1%)
None	7 (18·4%)	13 (28·9%)
History of immunization of household contacts with OPV	30 (78·9%)	28 (62·2%)

(type I, LS-c, 2ab/KP_4; type II P712, ch 2ab/KP_4; type III Leon $12a_1b/KP^5$) (i.e. vaccine at SO + 3). Thus passage levels of both monkey kidney and human diploid cell vaccines were equivalent. Both vaccines complied with the Compendium of Licensing Requirements for the Manufacture of Biological Medicinal Products for oral polio vaccine (Her Majesty's Stationery Office, 1977) and contained $\geqslant 10^{6\cdot0}$ TCID 50 type I, $\geqslant 10^{5\cdot0}$ TCID 50 type II and $\geqslant 10^{5\cdot5}$ TCID 50 type III virus per dose. After vaccination the potency of both vaccines was confirmed by re-titration of vaccine returned from each vaccination centre. All three types of each vaccine were found to be satisfactory in neurovirulence tests in monkeys at The Wellcome Research Laboratories, Beckenham.

Serology

Sera were titrated for neutralizing antibody to poliomyelitis types I, II and III. Serial two-fold dilutions, commencing at 1/4 (final 1/8), of pre- and post-vaccination sera were tested, in parallel, against an equal volume of virus at approximately 100 TCID 50 per 0·1 ml. Three hours at 36·5 °C were allowed for neutralization and residual unneutralized virus was detected using *Vero* cells in a microtitre system. The WHO International Standard Poliomyelitis Antisera were included in each test and the concentrations of antibody in test sera were calculated as described by Perkins & Evans (1959). Results were expressed in terms of international units per ml.

Virus isolation procedure

Faecal samples were collected in small sealable plastic buckets which were triple wrapped in polythene bags for safety. All specimens were stored at −20 °C until investigated.

1–2 g of faeces were transferred to a sterile universal bottle containing approximately ten 5 mm diameter glass beads. The material was made up to 10% (w/v) with Earle's balanced salt solution (BSS) containing 2·2 g/l sodium bicarbonate; penicillin 2000 i.u./ml; streptomycin 1000 i.u./ml and amphotericin B ('Fungizone' – E. R. Squibb and Sons) 25 μg/ml. The mixture was shaken to produce an evenly dispersed suspension and then allowed to stand for several minutes in an

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ice tray to allow any large particulate matter to settle. The supernatant was removed and centrifuged at approximately 2500 *g* for 20 min at 4 °C in an MSE Mistral 6L machine. The resultant extract was divided into three portions, one for immediate investigation and two for storage at −20 °C for repeat testing if required.

1 ml of extract was inoculated onto each of two confluent cultures of *Vero* cells in 25 cm^2 flasks. After allowing adsorption for 1 h at 36·5 °C, the inocula were removed and the cultures maintained with Medium 199 containing 2 % fetal calf serum, 1·76 g/l sodium bicarbonate, 1000 i.u./ml penicillin, 500 i.u./ml streptomycin and 5 μg/ml amphotericin B. The cultures were examined daily and harvested when extensive cytopathic effects (c.p.e.) became evident. Whilst cultures remained negative the maintenance medium was changed periodically. In some tests the substrate did not maintain for the intended 14 days but in no case was incubation shorter than 8 days. It was found that positive samples were usually apparent by the fourth day. The unclarified harvest fluid was stored at −20 °C in small volumes. For subsequent identity and marker testing each sample was clarified by low speed centrifugation.

Identification of viruses

Viruses were identified using commercially available polio neutralizing sera (Wellcome Reagents Limited). A divalent, trivalent serum pool technique was used with *Vero* cells as substrate.

The sample was diluted 10^{-2} and 10^{-5} in Sabin's No. 3 medium (Earle's BSS, 0·5 % lactalbumin hydrolysate brought to neutral pH by the addition of sodium hydroxide, 2·2 g/l sodium bicarbonate). Each dilution was mixed with an equal volume of divalent or trivalent polioantiserum and incubated for 1 h at 37 °C. The mixtures were then inoculated into *Vero* cultures in FB-16-24-TC plates (Flow Laboratories Limited) at 36·5 °C. The tests were first read 24 h after c.p.e. appeared in control cultures inoculated with 'un-neutralized' isolate and were finally read 8 days after inoculation when the identity of viruses present in the isolate was determined.

Marker tests

Reproductive capacity temperature (r.c.t./40) marker

All monovalent viruses were examined in r.c.t./40 marker tests in a microtitre system using *Vero* cells as the substrate.

Serial tenfold dilutions of the sample were prepared in Medium 199 containing 4 % fetal calf serum; 1·76 g/l sodium bicarbonate; 200 i.u./ml penicillin and 100 i.u./ml streptomycin. Each dilution was inoculated into 24 replicate microtitre wells (eight on each of three plates) containing 24- to 48-hour-old confluent *Vero* monolayers. After allowing 1 h at 36 °C for virus adsorption, the plates were divided into three groups for incubation at either 36·0°, 39·4° or 40·0 °C (40·3 °C when the virus under examination was type III). Appropriate r.c.t./40+ and r.c.t./40- controls were included in each test. Specific polio cytopathic effects were read 7 days after inoculation and titres calculated by the method of Karber (1931).

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'd' marker

Some of the monovalent viruses were also examined in 'd' marker tests using *Vero* cells grown in plastic tissue culture flasks (75 cm^2 surface area) as substrate. Virus growth characteristics, in terms of plaque numbers and plaque size, were investigated at bicarbonate concentrations of 0·22 % and 0·055 %.

Dilutions of the sample were prepared in Medium 199 containing 2·2 g/l sodium bicarbonate. Cultures for inoculation were pre-washed with virus diluent and 0·1 ml of two selected dilutions at an 0·3 Log_{10} interval, were allowed to adsorb to three *Vero* monolayers for 1 h at 37 °C. The dilutions were selected to produce approximately 25 plaques per flask at the higher bicarbonate concentration, and were reduced by 2·0 Log_{10} for the lower bicarbonate concentration. Following absorption the cultures were overlayed with 30 ml 1·5 % Difco Noble agar containing Medium 199, fetal calf serum, 1/40000 neutral red and sodium bicarbonate at final concentrations of either 0·22 % or 0·055 %. The cultures were incubated inverted at 36·5 °C and readings of plaque numbers and size made at daily intervals from the third to the sixth day after inoculation. Appropriate 'd' +ve and 'd' −ve controls were included in each test.

RESULTS

Vaccination history and detectable antibody

Twenty (24·1 %) of the 83 volunteers gave no previous history of vaccination with either OPV or IPV and 31 (37·3 %) reported that they had received IPV only, although with one exception this was not more recently than 1965. Twenty (24.1 %) volunteers had received OPV and 12 (14·5%) both IPV and OPV (Table 1). It is perhaps not surprising that the mean age for those with no previous history of vaccination (44·4 years) is significantly greater than for other groups (31·1 years), $P < 0·01$.

Nineteen (22·9 %) volunteers were without neutralizing antibody to one or more types at levels of 0·15 i.u./ml for types I and II and 0·1 i.u./ml for type III. Eleven volunteers were without detectable antibody to a single poliomyelitis type, 6 to two types and 2 to all three types. Nine of these 19 volunteers gave no previous history of vaccination and 6, 3 and 1 respectively histories of vaccination with OPV, IPV, and both IPV and OPV. Table 2 shows the correlation for each poliomyelitis type between vaccination history and the concentration of neutralizing antibody. Each volunteer's vaccination history was considered three times in relation to the concentration of antibody for each poliomyelitis type and the results summed. An inverse relationship was established between concentration of antibody and the absence of a previous history of vaccination. Some less definite relationships were also found with concentration of antibody and vaccination histories with both IPV and OPV and for OPV alone. The latter was clearer if only volunteers who had received two or more doses of OPV since 1965 were considered.

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Table 2. *Serum neutralizing antibody concentrations (i.u./ml)*

	< 0·15 Types I and II < 0·10 Type III	⩾ 0·15–< 1·5 Types I and II ⩾ 0·1–< 0·6 Type III	⩾ 1·5–< 5·0 Types I and II ⩾ 0·6–< 3·0 Type III	⩾ 5·0 Types I and II ⩾ 3·0 Type III
Total results*	29	55	83	82
Number of volunteers giving for each type				
No history of previous vaccination	18 (62 %)	12 (22 %)	19 (23 %)	11 (13 %)
History of vaccination with				
IPV and OPV	1 (3 %)	2 (4 %)	17 (20 %)	16 (20 %)
OPV alone	6 (21 %)	11 (20 %)	19 (23 %)	24 (29 %)
⩾ 2 doses since 1965	0 (0 %)	2 (4 %)	7 (8 %)	14 (17 %)
IPV alone	4 (14 %)	30 (55 %)	28 (34 %)	31 (37 %)
⩾ 3 doses	1 (3 %)	18 (33 %)	16 (19 %)	17 (21 %)

* Each serum tested against all three types of poliomyelitis. Thus each volunteer's vaccination history is triplicated. Number of volunteers giving vaccination history for each type as a percentage of total results is shown in parentheses.

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Response to vaccination

Six weeks after vaccination fourfold or greater increases in antibody concentration to one or more types were obtained in 53 (63·9%) volunteers. Increases in antibody concentration were found for all those with neutralizing antibody concentrations before vaccination of < 0·15 i.u./ml against types I and II and for five of 12 volunteers with neutralizing antibody concentrations of < 0·1 i.u./ml against type III (Table 3). However, all 7 subjects who failed to develop a significant antibody response to type III exhibited a fourfold or greater antibody response to types I and/or II. Furthermore, type III virus was recovered for 1–5 weeks after vaccination from five of the seven subjects from whom faecal samples were obtained.

The development of fourfold or greater antibody responses was inversely related to the concentration of antibody present before vaccination. Thus a majority of subjects with antibody concentrations before vaccination of ⩾ 0·15 to < 1·5 i.u./ml for types I and II and ⩾ 0·1 to < 0·6 i.u./ml for type III and a minority of subjects with higher antibody concentrations before vaccination developed fourfold or greater antibody responses. Significant differences in responses to monkey kidney and human diploid grown vaccines were not found.

Virus shedding

Faecal samples were collected from 58 volunteers. Two volunteers did not provide samples for 1 week, and one volunteer for 2 weeks. In addition the majority of samples from two other volunteers were cytotoxic to tissue culture. Forty-three (76·8%) of 56 volunteers shed virus 1 week after vaccination and thereafter the percentage declined until at 6 weeks virus was recovered from only six (10·5%) of the 57 volunteers studied. Indeed, no virus was recovered at any time during the 6-week surveillance period from only ten volunteers, and eight of these failed to show a fourfold or greater antibody response to any poliomyelitis type after vaccination. Both for monkey kidney and human diploid cell vaccines the isolation rates for type III were somewhat greater than for other types although the serological responses to type III were fewer (Tables 3 and 4). Thus while virus was recovered from 89% of those exhibiting a fourfold or greater increase in antibody concentration to each type after vaccination, virus was also recovered from 20·8%, 54·5% and 62·9% respectively of volunteers who did not show a fourfold or greater antibody response to type I, type II and type III. Virus shedding without a fourfold or greater antibody response was also more common in those with a history of previous OPV (5 of 17 (29·4%)) than in the other groups combined (4 of 41 (9·8%)), although the difference was not statistically significant. The extent to which virus was recovered from faecal samples after vaccination with monkey kidney and human diploid cell grown vaccine was remarkably similar.

Marker tests

No type I monovalent strains were isolated. Six monovalent type II and 21 monovalent type III strains from a total of 18 volunteers were examined in the

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Table 3. *Antibody responses to vaccination – serum neutralizing antibody concentrations (i.u./ml)*

Antibody concentration before vaccination	Monkey kidney vaccine: Sero-conversion rate	Monkey kidney vaccine: Mean pre-vaccination antibody concentration	Monkey kidney vaccine: Mean 6-week post-vaccination antibody concentration	Human diploid vaccine: Sero-conversion rate	Human diploid vaccine: Mean pre-vaccination antibody concentration	Human diploid vaccine: Mean 6-week post-vaccination antibody concentration
Type I						
< 0·15	5/5	—	14·0	6/6	—	20·7
⩾ 0·15–<1·5	6/8	0·66	21·1	7/8	0·79	28·6
⩾ 1·5–< 5·0	3/8	3·18	24·2	6/17	3·47	19·9
⩾ 5·0	0/17	18·50	21·2	3/14	17·83	33·9
Total	14/38 (36·8%)			22/45 (48·9%)		
Type II						
< 0·15	3/3	—	6·0	3/3	—	3·8
⩾ 0·15–< 1·5	9/15	0·7	13·6	11/12	0·8	13·2
⩾ 1·5–< 5·0	0/8	3·0	4·1	8/15	2·7	34·4
⩾ 5·0	2/12	16·8	29·3	1/15	13·8	22·2
Total	14/38 (36·8%)			23/45 (51·1%)		
Type III						
< 0·1	4/8	—	3·1 (6 subjects)*	1/4	—	4·8 (2 subjects)*
⩾ 0·1–< 0·6	2/4	0·15	1·2	7/8	0·3	6·2
⩾ 0·6–< 3·0	5/18	1·5	4·7	5/17	1·5	4·9
⩾ 3·0	0/8	3·3	9·3	0/16	7·5	9·8
Total	11/38 (28·9%)			13/45 (28·9%)		

* Subjects with titres ⩾ 0·1 included only.

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Table 4. *Percentage recovery of virus from faecal samples*

Weeks after administration of vaccine	Monkey kidney vaccine			Human diploid vaccine		
	Type 1	Type II	Type III	Type I	Type II	Type III
1	48·1%	59·3%	63·0%	55·2%	75·9%	68·9%
2	83·3%	25·9%	51·9%	28·6%	39·2%	46·4%
3	11·1%	14·8%	29·6%	14·3%	14·3%	28·6%
4	15·4%	7·7%	38·5%	13·3%	13·3%	23·3%
5	11·1%	3·7%	18·5%	7·2%	10·7%	17·9%
6	3·7%	3·7%	11·1%	3·3%	6·7%	6·7%
Total recovery of virus from faecal samples	33/161 (20·5%)	31/161 (19·3%)	57/161 (35·4%)	35/173 (20·2%)	46/173 (26·6%)	55/173 (31·8%)

Table 5. *Results of reproductive capacity temperature (r.c.t./40) marker tests on monovalent isolates*

Week isolate recovered after vaccination	Type II isolates					
	Monkey kidney, 2 studied			Human diploid, 4 studied		
	t − ve	t ±	t + ve	t − ve	t ±	t + ve
1	a	b	—	c, d, e		
6	—	—	—	c		
	Type III isolates					
	Monkey kidney, 12 studied			Human diploid, 9 studied		
	t − ve	t ±	t + ve	t − ve	t ±	t + ve
1	—	f	—	—	g	
2	h	i, m	—	j	k, l	
3	n	o	—	—	p	
4	m	f, q	—	k	—	
5	n	—	—	—	g	l
6	i, s	—	—	—	j	

Each letter refers to a single volunteer.
Two isolates were collected from volunteers c, f, g, i, j, k, l, m and n.

r.c.t./40 marker test (Table 5). These strains were derived from eight volunteers vaccinated with human diploid and ten volunteers vaccinated with monkey kidney grown vaccine.

Five of six type II, and eight of 21 type III viruses were found to be t negative in that there was more than a 10^{-3} reduction in the titre of virus growth at 39·4 °C in comparison with 36 °C and more than a 10^{-5} reduction in growth at temperatures of 40 °C (type II) or 40·3 °C (type III) compared with 36 °C (WHO Expert Committee on Biological Standardization, 1972). One of two type II strains from volunteers vaccinated with monkey kidney grown vaccine was found to have an intermediate t marker as were six of 12 type III viruses isolated and six of nine type III viruses isolated from two groups of volunteers who had received vaccine grown in monkey kidney and human diploid cells respectively. One of two type III viruses isolated from a volunteer vaccinated with human diploid grown vaccine

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was t positive. The other virus isolated from this volunteer was of intermediate t characteristic.

Five and six viruses isolated respectively from volunteers vaccinated with human diploid and monkey kidney grown vaccines were also studied in the 'd' marker test. No loss of 'd' marker was found and no differences were shown between vaccines in the reductions of titre or plaque size with reduced bicarbonate concentration.

DISCUSSION

The recent increase in the number of cases of paralytic poliomyelitis in the United Kingdom is of concern. It is notable that 53 (63·9 %) of our 85 volunteers exhibited a fourfold or greater antibody response to vaccination while virus was recovered from 48 (82·8 %) of 58 volunteers providing faecal samples. All these vaccinees, however, would not be at risk from paralytic poliomyelitis since all but 19 (22·9 %) had detectable amounts of antibody to all poliomyelitis types and could therefore be expected to be protected and to develop a rapid secondary antibody response to infection (Salk & Salk, 1977). Mortimer & Cunningham (1975) found that 12 % of 5- to 14-year-old children were without detectable antibody to one or more types. Smith *et al.* (1976) found that 14·4 % of 16- to 18-year-old police cadets were without antibody to at least one type. Our results are not dissimilar. They illustrate the possibilities for spread of infection with wild poliomyelitis through a population. This is significant since poliomyelitis virus strains with fully virulent characteristics are still isolated from specimens submitted to diagnostic laboratories (Cossart, 1977). It must be admitted, however, that the titre of virus in a dose of vaccine was established to elicit a secondary antibody response and is much greater than is necessary for primary immunization (Hobson *et al.* 1962; Beale, 1969). Thus some of the volunteers who were infected by a dose of vaccine containing a large dose of virus may not have been infected by a smaller challenge dose of wild virus.

The monkey kidney and human diploid cell vaccines compared here appear to be equivalent in the rate and level of antibody responses elicited to all three poliomyelitis types and in the extent of virus shedding. With both vaccines virus shedding of type III predominated over types I and II. No volunteer was left without detectable circulating neutralizing antibody to types I and II but seven (8·4 %) had antibody concentrations of < 0·1 i.u./ml to type III. Nevertheless, of these seven volunteers all five who were studied shed virus for 1–5 weeks after vaccination in the absence of an antibody response. There is, therefore, evidence of virus replication in the intestine and development of a local IgA antibody response in these volunteers may be presumed.

It is known that following human passage vaccine strains may lose t – ve marker characteristics and increase in neurovirulence (MacLeod, 1966; Furesz *et al.* 1966). No significant difference in loss of the t – ve characteristic of type III isolates recovered from volunteers vaccinated with vaccines grown in monkey kidney or human diploid cells was shown in our study. This is in agreement with the results

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of similar studies carried out with vaccines containing other poliomyelitis strains grown in monkey kidney or human diploid cell substrates (Ikic *et al.* 1970*a*, *b*).

In vitro marker tests with their wide limits of error are not the best method for determining changes in Sabin vaccine strains but the surer alternative method of neurovirulence tests in monkeys was precluded in our study on the grounds of the high cost of each test and the world shortage of suitable monkeys.

The use of human diploid cells in place of monkey kidney cells as a virus substrate was found to be satisfactory and provides advantages in standardization of virus substrate, less risk of contamination with extraneous agents and freedom from foreign animal protein.

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REFERENCES

BEALE, A. J. (1969). Immunization against poliomyelitis. *British Medical Bulletin* **25**, 148.

COMPENDIUM OF LICENSING REQUIREMENTS FOR THE MANUFACTURE OF BIOLOGICAL MEDICINAL PRODUCTS (1977). Her Majesty's Stationery Office.

COSSART, Y. E. (1977). Evolution of poliovirus since introduction of attenuated vaccine. *British Medical Journal* i, 1621.

DEPARTMENT OF HEALTH AND SOCIAL SECURITY (1976). Health and Personal Social Services Statistics for England. Her Majesty's Stationery Office.

FURESZ, J., ARMSTRONG, R. E., MOREAU, P., YAROSH, W. & NAGLER, F. P. (1966). Antigenic studies on Sabin Types 1 and 3 poliovaccine virus during 1 to 7 passage in the human intestinal tract. *American Journal of Epidemiology* **83**, 501.

HAYFLICK, L. & MOORHEAD, P. S. (1961). The serial cultivation of human diploid cell strains. *Experimental Cell Research* **25**, 585.

HOBSON, D., HOSKINS, J. M., LANE, C. A., ELLIOTT, R. W., PERKINS, F. T. & YETTS, R. (1962). Effect of a single dose of oral poliovirus vaccine in persons previously immunized with inactivated vaccine. *British Medical Journal* ii, 145.

IKIC, D., MANHALTER, T., HRABAR, A., JANCIKIC, B., JUZBASIC, M., LULIC, V. & TURNER, V. (1970*a*). Vaccination against poliomyelitis with live poliovaccine prepared in MKTC and HDC in S.R. Croatia. *Proceedings of the Symposium on Human Diploid Cells*. Yugoslav Academy of Sciences and Arts, Zagreb, p. 93.

IKIC, D., HRABAR, A., MANHALTER, T., JANCIKIC, B., JUZBASIC, M., LULIC, V. & RADMAN, V. (1970*b*). Safety of WM-3 poliovirus vaccine. *Proceedings of the Symposium on Human Diploid Cells*. Yugoslav Academy of Sciences and Arts, Zagreb. p. 105.

KARBER, G. (1931). Beitrag zur kooektiven Behandlung pharmakologischer Reinhenversuche. *Archiv für experimentelle Pathologie und Pharmakologie* **162**, 480.

MACLEOD, D. R. E. (1966). The neurovirulence of the type 3 Sabin vaccine strain after passage in infants. *Canadian Journal of Public Health* **57**, 37.

MORTIMER, P. P. & CUNNINGHAM, P. (1975). Sero-immunity to poliovirus in children and young women: England 1972–4. *Journal of Hygiene* **74**, 283.

OFFICE OF POPULATION CENSUSES AND SURVEYS (1975). Statistics of Infectious Disease 1975, England and Wales. Series MB2, No. 2. Her Majesty's Stationery Office.

OFFICE OF POPULATION CENSUSES AND SURVEYS (1978). Monitor for Quarter Ending March. Ref. MB2 78/3. Her Majesty's Stationery Office.

PERKINS, F. T. & EVANS, D. G. (1959). British standard poliomyelitis antisera types 1, 2 and 3. *British Medical Journal* i, 1549.

SABIN, A. B. (1957). Properties and behaviour of orally administered attenuated poliovirus vaccine. *Journal of the American Medical Association* **164**, 1216.

SABIN, A. B. (1959). Present position of immunization against poliomyelitis with live virus vaccines. *British Medical Journal* i, 663.

https://doi.org/10.1017/S0022172400026474 Published online by Cambridge University Press

Salk, J. & Salk, J. (1977). Control of influenza and poliomyelitis with killed virus vaccines. *Science* **195**, 834.

Smith, J. W. G., Lee, J. A., Fletcher, W. B., Morris, C. A., Parker, D. A., Yetts, R., Magrath, D. I. & Perkins, F. T. (1976). The response to oral poliovaccine in persons aged 16–18 years. *Journal of Hygiene* **76**, 235.

W.H.O. Expert Committee on Biological Standardization (1972). Twenty-fourth Report. *World Health Organisation Technical Report Series*, no. 486.

https://doi.org/10.1017/S0022172400026474 Published online by Cambridge University Press