Observations on a poliovirus variant

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INTRODUCTION

Between the months of March 1956 and September 1957 a survey was conducted in Dunedin, New Zealand, to try to discover the frequency with which adenoviruses could be isolated from the community (Abel, 1957). One hundred and thirty-one tonsils and 85 adenoids from routine tonsillectomies and adenoidectomies were examined as well as nine throat washings which were received during an epidemic of upper respiratory tract infection in 1957, and a few other miscellaneous clinical specimens. During the greater part of the time no isolations at all were made, but in the months of July and August 1957 four viruses were isolated. In the second week of July an eye-swab from a child suffering from conjunctivitis yielded an agent which caused cytopathic changes in HeLa cells. Two weeks later similar agents were isolated from tonsillar tissue of a child and from throat washings of an adult suffering from an upper respiratory tract infection. In the third week of August a further isolation was made from faeces of a child suffering from a biliary obstruction. The survey was terminated early in September 1957.

A close relationship amongst the four agents was demonstrated by cross-neutralization tests and complement-fixation tests. The latter technique was also used to show that they were not members of the adenovirus group. Preliminary cross-neutralization tests showed no cross-reaction with ECHO virus Types 1–14 or poliovirus Types 1 and 2. They did, however, reveal a serological relationship with poliovirus Type 3. As there was no clinical poliomyelitis in the district at the time, it was thought possible that the group of agents isolated might belong to a naturally occurring avirulent strain of poliovirus Type 3. For this reason the virus isolated from the eye (FES) was compared with Saukett, the standard strain of this type used in this laboratory.

MATERIALS AND METHODS

Tissue cultures

The HeLa strain of epidermoid carcinoma cells was used, both to prepare stocks of the viruses and to titrate virus preparations. The cells were originally obtained from the Virus Unit of the Auckland Hospital and were serially propagated in a medium consisting of 10 % heated rabbit serum and 0·25 % lactalbumin hydrolysate in Hanks' balanced salt solution containing 0·1 % sodium bircarbonate. The medium also contained 100 units of penicillin and 100 μg. of streptomycin

in each ml. The maintenance medium contained 3 % rabbit serum instead of 10 %.

Tissue cultures were prepared from rhesus monkey kidneys using the method and medium described by Melnick (1956).

Viruses

FES. The origin of this virus has been described in the introduction.

Saukett strain of poliovirus Type 3. This was obtained from the Virus Unit of the Auckland Hospital as the 20th HeLa cell tissue culture passage.

Pure cultures of both viruses were obtained by two cycles of passaging from a single plaque on a HeLa cell monolayer tissue culture.

The virus concentration of any preparation was determined by inoculating 0·1 ml. of serial dilutions into each of five tubes which had been seeded 2 days previously with 150,000 HeLa cells. After making the volume up to 1 ml. the tubes were incubated at 37° C. for 6 days, when they were examined microscopically for tissue degeneration. A tube was regarded as infected by virus if 50 % or more of the cells showed definite cytopathic changes. The dilution containing 1 TCD50 was calculated by the method of Reed & Muench (1938).

Monkey inoculation

0·25 ml. of virus-infected tissue culture fluid was injected intracerebrally into rhesus monkeys. Animals were observed daily for evidence of paralysis and rectal temperatures were taken twice daily for the first 12 days after injection and then once daily until the 30th day.

Filtration

Virus-infected tissue culture fluids were clarified by centrifigation and filtration through a Gradocol membrane with an average pore diameter (A.P.D.) of 730 mμ. 5 ml. aliquots were filtered through Gradocol membranes of various porosities under a nitrogen pressure of 20 lb. per sq. in. The effluent from each filter was collected for virus titration.

The Gradocol membranes were obtained from the Wright–Fleming Institute, St Mary's Hospital, London.

Ultracentrifugation

Virus-infected tissue culture fluids were clarified by centrifugation at 2000 r.p.m. for 10 min. The titre of these fluids was used as the basis of comparison for the supernatants. The fluids were centrifuged at 32,500 r.p.m. for different periods of time in the 40 rotor of a Spinco model L preparative ultracentrifuge. At the end of each run the top 1 ml. of supernatant fluid was removed and titrated for virus content.

Neutralization

Sera (hyperimmune rabbit) were diluted 1:10 in maintenance medium (M.M.) and heated at 56° C. for 30 min. They were then serially diluted in M.M. in four-fold

steps to 1:2560. The serum dilutions were mixed with an equal volume of virus diluted to contain 1000 TCD 50 in each ml. Neutralization was allowed to proceed for 60 min. at room-temperature after which 0·2 ml. of the virus serum mixture was added to each of four or five HeLa cell culture tubes so that each tube received 100 TCD 50 of virus. The fluid volume was made up to 1 ml. with M.M. Controls were set up including a titration of the virus preparation used in the test, and M.M. without virus. The cultures were examined microscopically for tissue degeneration after 3 or 4 days at 37° C. A preliminary test showed that the sera alone did not cause cytopathic changes at a dilution of 1:10.

The serological relationship between FES and Saukett was assessed by calculation of the antigenic ratio (R) (Archetti & Horsfall, 1950). This is the geometric mean of 'r_1', which is obtained by dividing the heterologous titre of virus 2 by the homologous titre of virus 1, and 'r_2', which is obtained by dividing the heterologous titre of virus 1 by the homologous titre of virus 2 (i.e. $R = \sqrt{r_1 r_2}$).

Temperature stability

1 ml. ampoules of infected tissue culture fluid were submerged in a water-bath at 44° C. After 2 hr. they were removed and the contents titrated for virus infectivity. The contents of unheated ampoules were titrated as controls.

Ether sensitivity

The method of Andrewes & Horstmann (1949) was used.

RESULTS

Growth in tissue cultures

FES virus grew in both HeLa cells and monkey kidney cells causing a cytopathic effect (CPE) which differed in no way from the CPE caused by Saukett virus. When sheets of HeLa cells and monkey kidney cells were infected with a suitable concentration of FES virus plaques developed upon subsequent incubation under maintenance medium containing agar and neutral red. The plaques produced by FES virus and Saukett virus were similar in size, morphology and time of appearance.

Pathogenicity for monkeys

Two rhesus monkeys were injected intracerebrally with FES virus, one receiving 25,000 TCD 50 and the other 25 TCD 50. The recipient of the larger dose developed a pyrexia lasting from the 5th to the 8th day. The animal had slight tremors during the pyrexia but there was no limb weakness or paralysis. FES neutralizing antibodies were present in the serum 3 weeks after injection.

The temperature of the animal receiving 25 TCD 50 of virus remained normal and no neutralizing antibodies appeared.

Four weeks after injection with FES virus each animal was challenged with 25,000 TCD 50 of Saukett virus administered intracerebrally. The monkey with

neutralizing antibodies against FES virus remained normal. The temperature of the one lacking FES antibodies rose above normal on the 4th day and remained elevated for 7 days. During this period the animal had general tremors but there was no evidence of paralysis or limb weakness at any stage.

Filtration and ultracentrifugation

No difference in the properties of FES and Saukett viruses could be demonstrated by filtration through Gradocol membranes or by ultracentrifugation. By the former technique the size of both particles was estimated to be between 17 and 32 mμ and by the latter between 30 and 35 mμ.

Table 1. *Cross-neutralization tests with FES and Saukett viruses*

		Virus		Heterologous titre	
				Homologous titre	
Expt.	Serum	FES	Saukett	titre	R
1	FES	320*	80	1/4	0·22
	Saukett	10	50	1/5	
2	FES	320	640	2	0·35
	Saukett	20	320	1/16	
3	FES	250	250	1	0·45
	Saukett	10	50	1/5	

* Reciprocal of serum dilution protecting against 100 TCD 50 of virus.

Table 2. *Inactivation of FES and Saukett viruses at 44° C.*

	Virus infectivity (Neg. Log TCD 50)			
	FES		Saukett	
Time (hr.)	Expt. 1	Expt. 2	Expt. 1	Expt. 2
0	5·2	4·5	5·2	4·7
0·5	4·8	—	3·8	—
1	4·5	—	3·2	—
2	4·4	3·4	2·2	2·7

Neutralization

The results of a series of cross-neutralization tests in which FES antiserum and Saukett antiserum were tested against both viruses are shown in Table 1. The same sera were used in all three experiments, but the virus preparations were different for each one.

Temperature stability

When FES virus and Saukett virus were tested for their stability at 44° C. over a period of 2 hr. the infectivity titre of FES dropped from $10^{5·2}$ to $10^{4·4}$, while that of Saukett dropped from $10^{5·2}$ to $10^{2·2}$. This represents a survival of 17 % of FES

virus, but only 0·1 % of Saukett virus. Details of two experiments on temperature stability are given in Table 2.

Ether sensitivity

When FES virus and Saukett virus were tested for their sensitivity to ether it was found that they both resisted inactivation by this chemical.

DISCUSSION

The results of the reciprocal neutralization tests and the monkey protection test indicate the close antigenic relationship between the FES and Saukett viruses and strongly suggest that FES is a Type 3 poliovirus. The accumulated data on particle size, type of cytopathic effect on HeLa and monkey kidney cells, plaque formation on monolayer cultures of these cells and resistance to ether support this contention.

Dubes, Archetti & Wenner (1959) when investigating antigenic variation amongst Type 3 polioviruses found intra-strain variations, in which the antigenic ration (R) ranged from 0·56 to 1·22 with an average of 0·86, and inter-strain variations in which the range of R was from 0·2 to 1·61 with a mean of 0·49. The values of R obtained in the three cross-neutralization experiments between FES and Saukett viruses (0·22, 0·35 and 0·45) are all less than the minimum value obtained for intra-strain variation, but are within the range of inter-strain variation. While it seems justifiable to regard the FES virus as a Type 3 poliovirus, the antigenic difference between it and the Saukett strain is greater than would be expected if they had represented two isolations from separate patients in an outbreak of poliomyelitis caused by Type 3 virus. The difference in stability at 44° C. between the FES and Saukett viruses is an additional indication that the two strains are not identical.

The failure of 25,000 TCD 50 of FES virus administered intracerebrally to cause paralysis in a monkey indicates that it is a strain of low neuropathogenicity for primates. The isolation from apparently healthy children of a number of poliovirus strains showing a wide spectrum of neurotropism has been reported by Sabin (1957).

From the experimental evidence it is clear that FES virus is a Type 3 poliovirus with reduced neurotropism for primates. It shows a slight antigenic difference from the Saukett strain of Type 3 poliovirus, and also differs from it in temperature stability.

The occurrence of this virus in the eye of a child suffering from conjunctivitis does not necessarily mean that it was the aetiological agent of the clinical condition. In view of the fact that there are no reports of a poliovirus or any other member of the enterovirus family ever having been isolated from material from an eye-swab, the most likely explanation is that the virus had been mechanically transferred to the eye from elsewhere. The child was experiencing an illness not unlike the undifferentiated febrile illness which poliovirus infection can cause (Melnick & Sabin, 1959). At the same time she was suffering from an irritant conjunctivitis.

It is not difficult to imagine the ease with which virus-containing material could be transferred to the eye on soiled fingers in a 3-year-old child's efforts to ease the irritation by rubbing.

The clinical specimens from which the other three virus isolations were made originated in the pharynx (tonsil tissue and throat washings) and the alimentary canal (faeces). Both of these sites provide the material of choice in attempting the isolation of polioviruses either from clinical cases or carriers. Experimental work has shown that peripheral trauma is a predisposing factor in central nervous systen infection with polioviruses (Bodian, 1959). From this it would be expected that the injury associated with the surgical removal of a tonsil carrying poliovirus should provide conditions under which even a moderately neurotropic virus would be able to invade the central nervous system. The fact that the patient did not develop clinical poliomyelitis is a further indication that these poliovirus strains had reduced neurotropism for primates.

There is experimental evidence that the different types of poliovirus interfere with each other (Koprowski, 1955; Sabin, 1957). Evidence suggesting that some such interference phenomenon might be effective under natural conditions in preventing the establishment of a virulent poliovirus in the alimentary canal is found in a report by Hale, Doraisingham, Kanagaratnam, Leong & Monteiro (1959). They report that, during an epidemic caused by Type 1 poliovirus, the number of cases in a group receiving Sabin attenuated Type 2 virus by mouth was very much lower than that in the rest of the population of comparable age at risk. It would seem likely that in this case virus interference was of more importance than immunization.

The fact that four different virus isolations were made from a limited number of clinical specimens in a brief period suggests that during that time the population was heavily infected with this non-neurotropic strain of poliovirus Type 3 and that despite its low virulence the strain has a high infectivity. This period of infection may not have been as brief as the isolations suggest, because the last isolation was made only a week or so before the survey was terminated in September 1957. The presence of this non-neurotropic poliovirus Type 3 in the community would have the same effect as a single dose of a univalent live poliovirus vaccine. There would be stimulation of specific antibody production and the presence of the virus in the alimentary canal would reduce the chances of a potentially pathogenic poliovirus strain establishing itself there. The complete absence from the district of clinical cases of poliomyelitis between May 1957 and January 1958 could have been due to the presence of this virus.

SUMMARY

1. Some of the biological and physical properties of one member (FES) of an inter-related group of viruses were examined and compared with the Saukett strain of poliovirus Type 3.

2. The accumulated data of particle size, immunological relationship, and host tissue and cell tropism indicates that this is a naturally occurring non-neurotropic

strain of poliovirus Type 3. It shows a slight immunological difference from Saukett, and also differs from it in temperature stability.

3. The significance of four different isolations of this agent in a brief period is discussed.

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