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INTERNATIONAL COMPARISON OF SPECIES OF MONKEY USED FOR THE NEUROVIRULENCE TEST FOR ORAL POLIOMYELITIS VACCINE (*)

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The results of an international comparative study of the neurovirulence of oral poliomyelitis vaccines in three species of Old World Monkeys, viz. *Macacus irus* (cynomolgus), *M. mulatta* (rhesus) and *Cercopithecus aethiops* (vervet) are presented. The greatest sensitivity to Sabin poliovaccine types I and III was shown by cynomolgus monkeys, followed by rhesus and vervet monkeys, whilst vervet monkeys showed the greatest sensitivity to Sabin type II poliovirus. The biological basis for the apparently greater sensitivity of vervet monkeys to the type II poliovirus is not clear.

The results presented in this paper emphasize the desirability of using homotypic poliovirus reference strains for the neurovirulence test.

INTRODUCTION

There have been few comparative studies of the behaviour of the three poliovirus serotypes in different simian species.

The neurovirulence studies which led to the selection of the Sabin strains of poliovirus for production of live, attenuated vaccines were performed in rhesus monkeys and this species was initially adopted for routine neurovirulence assays of the vaccine (Murray, Kirschstein, Van Hoosier & Baron, 1959). A subsequent investigation of the response of five species of Old World Monkeys to intraspinal inoculation of type I and type III attenuated poliovirus indicated that patas

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was the most sensitive species, followed by cynomolgus, vervet, thesus and baboons in order of decreasing sensitivity (Boulger & Perkins, 1965), and an international comparative study (1973) showed that cynomolgus monkes were also more sensitive than rhesus monkeys to type I and type III attenuated poliovirus inoculated directly into the thalamus.

The purpose of the present study was a direct comparison of the response of three species of monkey frequently used for the neurovirulence test-cynomolgus monkeys (*Macacus irus*), rhesus monkeys (*Macacus mulatta*) and vervet monkeys (*Cercopithecus aethiops*)—to the intraspinal and intracerebral inoculation of the three poliovirus serotypes.

MATERIALS AND METHODS

Three Institutes collaborated in the study:

(i) The National Institute for Biological Standards and Control, London, England.

(ii) The Razi State Institute for Vaccines and Sera Tehran Iran.

(iii) The National Institute for Communicable Diseases, Delhi India.

The various passage levels of the Sabin attenuated poliovirus strains in use for the production of oral poliomyelitis vaccine (OPV) are classified according to a system of strain notation (Sabin & Boulger 1973).

The reference virus suspensions for neurovirulence tests were prepared in London by serial passages of the Sabin Merck, Sharpe & Dohme attenuated polioviruses previously treated with SV40 antiserum in *Erythrocebus patas* monkey-kidney cell cultures and distributed to the Institutes in Delhi and Teheran. The Sabin Merck Sharpe & Dohme vaccine was designated SOM (SO+1) being derived from the Sabin original virus (SO) by one passage in rhesus monkey kidney cell cultures.

The harvests from the next passage (SO+2) were designated 69/141-type I, 69/142-type II and 69/143-type III (see Table I). A further serial passage (SO+3) was designated 69/301-type I, 69/302-type II and 69/303-type III. In London and in Teheran the neurovirulence tests were performed on the 69/301, 302 and 303 preparations but in Delhi the tests were performed on the 69/141-type I, 69/302-type II and 69/143-type III references.

The performance and histological assessment of the intraspinal and intracerebral tests were similar in Delhi, London and Teheran and followed the method of Boulger (1973). Only the monkey species differed, rhesus monkeys being used in Delhi, cynomolgus monkeys in London and vervet monkeys in Teheran.

The assessment of neurovirulence depended on the presence or absence of histological neuronal lesions in the central nervous system of the monkeys (Boulger, 1973).

Intracerebral test

The W.H.O. Technical Report Series (1972) recommends that in countries where reliance is placed mainly on the intracerebral (i.c.) test 30 monkeys are inoculated intrathalamically with undiluted vaccine. In other countries, where emphasis is placed mainly on the intraspinal (i.s.) test, the i.c. test is performed by inoculating two groups of ten monkeys, one with undiluted vaccine and the other with one tenfold dilution of vaccine. In the present study, undiluted vaccine was inoculated into groups of 30 monkeys in Delhi and Teheran, while in London two groups of ten monkeys were inoculated, one with undiluted vaccine and the other with a tenfold dilution of the vaccine. This accounts for the variation in numbers of intracerebrally inoculated monkeys between the three centres. The volume of inocula was 1.0 ml in the three laboratories, 0.5 ml being inoculated into each hemisphere.

The inoculation of 10^8, 10^7 or 10^6 TCD_{50} of attenuated virus into the thalamus produces so few specific lesions in the central nervous system that the calculation a of 50% endpoint is not possible. In order to assess the intracerebral tests presented in this paper a direct comparison was made between the specific histological lesions produced at sim ilar levels of the central nervous system by the inoculated poliovirus. In order to present the results in a concise form these are compared as thalamus and 'spread'. The thalmus level includes the site(s) of inoculation, while 'spread' is restricted to neuronal lesions in the lumbar cord (Table 5).

Intraspinal test

In additon to examining the lumbar cord for neuronal lesions, six levels of the 'brain' of each intraspinally inoculated animal were examined for neurcnal lesions as follows: cervical cord, medulla oblongata, pons and cerebellum, midbrain, thalamus and cortex. S milarly, 27 segments/regions/nuclei of the 'brain' were examined; cervical cord, red nucleus, reticular formation of medulla and pons, lateral vestibular nucleus, lateral reticular nucleus, dentate nucleus, emboliform/globose nuclei, fastigial nucleus, precentral gyrus, lateral cuneate nucleus, substantia nigra, inferior olivary nucleus, medial vestibular nucleus, tegmentum, thalamus, oculomotor/central gray matter, subthalmus/hypothalamus, globus pallidus/putamen/caudate nuclei, abducens/facial nuclei, hypoglossal/dorsal motor vagus nuclei, gracillis and cuneate nuclei, dorsal and ventral coch-

lear/pontile nuclei, ambiguus/spinal tract of trigeminal nerve nuclei, parietal/insular/temporal cortices of thalamus, postcentral gyrus, supraspinal/accessary nuclei and cerebellar cortex. For these calculations a neuronal lesion occurring in one hemisection was counted as present in the complete section.

Four parameters were employed in order to assess the degree of virus activity and hence the sensitivity of the monkeys:

(i) the lumbar neuronal lesion 50% endpoint (LNLD50);

(ii) the 'brain' neuronal lesion 50% endpoint (BNLD50);

(iii) the percentage of 'brain' levels showing neuronal lesions;

(iv) the percentage of 'brain' segments/regions/nuclei showing neuronal lesions.

The LNLD50 and BNLD50 were defined respectively as the number of tissue culture infective doses of virus (TCD) which were required to produce neuronal lesions in 50% of the intraspinally inoculated monkeys in the lumbar cord and 'brain'. These values were expressed as log10/0.2 ml vaccine (Delhi) or log10/0.1 ml vaccine (London and Teheran).

The use of the LNLD50 and BNLD50 alone ignores considerable histological information concerning the number of 'brain' levels affected in an individual monkey and the number of neuronal lesions present at each level. The degree of spread of virus activity to the 'brain' has therefore been considered (parameters (iii) and (iv)) in addition to the 50% endpoint (parameters (i) and (ii)).

The formulae utilized in computing the percentage of 'brain' levels and of 'brain' nuclei affected were:

Percentage of 'brain' levels with neuronal lesions

$$= \frac{\text{No. of actual positives}}{\text{No. of possible positives (6)} \times \text{no. of valid † monkeys}} \times 100,$$

Percentage of 'brain' nuclei with neuronal lesions

$$\frac{\text{No. of actual positives}}{\text{No. of possible positives (27)} \times \text{no. of valid † monkeys}} \times 100.$$

The volume of inocula for the intraspinal test was 0.1 ml in London and Teheran and 0.2 ml in Delhi.

† A monkey is regarded as valid when it survives a minimum of 15 days poss-inoculation and exhibits one or more inoculation sites in the grey matter of the lumbar cord. At the discretion of the histopahologist is may also be considered valid in the absence of a correctly placed inoculation if there is evidence of histoiogical poliomyelitis at multiple levels of the c:ntral nervous system.

RESULTS

The details of the neurovirulence test, the species of monkey and the passage levels of the Sabin reference poliovirus strains used in the three Institutes are presented in Table 1.

Table 2 shows the results of the i.s. tests performed. The histological poliomyelitis data are expressed as 50% lumbar and 'brain' endpoints. It is seen that the results obtained in Delhi and London (rhesus and cynomolgus monkeys) were similar for all three poliovirus serotypes. However, in Teheran, where vervet monkeys were used, the lumbar endopoints were higher than in Delhi and London, although this is not so for the 'brain' endpoints. These results suggest that the motor neurones of the lumbosacral cord of vervet monkeysare less sensitive to the Sabin vaccine strains than those of cynomolgus and rhesus monkeys.

The three sets of results are further compared in Tables 3 and 4 according to the distribution of neuronal poliomyelitis at the target levels of the 'brain. The spread of histological poliomyelitis recorded in the three Institutes using the SO+3 type II virus preparation was similar in Delhi and London but greater in the vervet monkeys used in Teheran. The increased sensitivity in vervet monkeys for type II poliovirus is further indicated by a lower BNLD50 in the Teheran results compared with those in Delhi and London (Table 2). In comparing the lesions produced by types I and III viruses it should be noted that in Delhi SO+2 material was used compared to SO+3 in London and Teheran. This introduces the possibility of the SO+2 material being slightly less neurovirulent, if it is accepted that a further passage of the virus is likely to produce greater virulence, especially in type II poliovirus (Boulger & Magrath, 1973). Bearing this possibility in mind it is apparent that cynomolgus monkeys are most sensitive to Sabin strains I and III and vervet monkeys least sensitive.

Table 5 shows the results of the intracerebral tests utilizing the numbers and percentages of monkeys with neuronal lesions in the thalamus and lubmar cord. The assessment of specific microscopic lesions in the thalamic region of the brain, i.e. at, and adjacent to, the sites of inoculation, is relatively imprecise due to the associated presence of histological changes directly attributable to injection trauma. However, the spread of neuronal lesions to the lumbar segments of the spinal cord after i.c. injection is readily recognizable and, as such, this criterion is a strict and precise indicator of neurovirulence. After the inoculation of SO+3 passage type II poliovirus neuronal lesions in the lumbar cord were most evident in the Teheran vervet monkeys. When the incidence of specific neuronal lesions in the lumbar cord produced by type I and type III poliovirus was assessed, cynomolgus monkeys were found to be the most sensitive of the three species of monkey, especially to type III poliovirus (even after allowance for the fact that these two serotypes were at the SO+2 passage level in Delhi).

TABLE 1. Details of the neurovirulence test and reference virus preparations in the three Institutes

Institute	Species of monkey	Volume of intraspinal inoculum (ml)	Volume of intracerebral inoculum (ml)	Name of reference virus (69/) Poliovirus serotype I	II	III	Passage level from Sabin Original Virus Poliovirus serotype I	II	III
London	*M. irus* (cynomolgus)	1 × 0·1	2 × 0·5	301	302	303	SO + 3	SO + 3	SO + 3
Delhi	*M. mulatta* (rhesus)	1 × 0·2	2 × 0·5	141	302	143	SO + 2	SO + 3	SO + 2
Teheran	*C. aethiops* (vervet)	1 × 0·1	2 × 0·5	301	302	303	SO + 3	SO + 3	SO + 3

Sabin Original Virus (SO) type I: L-Sc, 2ab/KP_2.
Sabin Original Virus (SO) type II: P712, Ch, 2ab/KP_2.
Sabin Original Virus (SO) type III: Leon $12a_1b/KP_3$.

TABLE 2. Summary results of intraspinal tests expressed as 50% endpoints

	Poliovirus serotype								
	I Institutes			II Institutes			III Institutes		
	London	Delhi	Teheran	London	Delhi	Teheran	London	Delhi	Teheran
Maximum dose of virus injected (log_{10})	6·3	5·9	6·3	6·1	7·1	6·2	6·3	6·8	6·2
No. of monkeys with valid injection	25	29	28	27	28	27	26	30	30
Proportion of levels with injection trauma	0·36	0·32	0·52	0·31	0·40	0·30	0·30	0·35	0·45
Histological neuronal poliomyelitis (log_{10})									
Lumber lesion 50% endpoints ($LNLD_{50}$)	3·3	2·9	>6·3	3·2	3·5	5·2	4·4	3·7	5·4
Brain lesion 50% endpoints ($BNLD_{50}$)	6·1	>5·9	>6·3	>6·1	6·4	5·8	5·7	5·8	>6·2

London and Delhi results: mean of three tests. Teheran results: mean of two tests.
Values expressed to: whole numbers for valid monkeys, first decimal places for virus doses, and second decimal places for injection trauma.

TABLE 3. Summary results of intraspinal tests showing percentages of 'target' levels with neuronal lesions

	Poliovirus serotype								
	I Institutes			II Institutes			III Institutes		
	London	Delhi	Teheran	London	Delhi	Teheran	London	Delhi	Teheran
Maximum dose of virus injected (log_{10})	6·3	5·9	6·3	6·1	7·1	6·2	6·3	6·8	6·2
No. of monkeys with valid injection	25	29	28	27	28	27	26	30	30
Cervical cord*	15·3	2·4	0·0	2·6	8·1	11·1	18·3	14·3	8·5
Medulla oblongata	5·3	1·0	0·0	3·7	1·1	7·4	16·7	3·3	6·8
Pons and cerebellum	13·4	1·0	0·0	8·6	7·1	9·3	23·3	16·7	3·4
Midbrain	8·1	1·0	0·0	14·1	10·6	14·8	23·3	14·3	5·1
Thalamus	0·0	0·0	0·0	0·0	0·0	0·0	10·5	1·0	0·0
Cerebral cortex	1·2	1·0	0·0	0·0	0·0	1·9	10·5	0·0	1·7
Percentage of positive levels of cervical cord and brain	6·1	1·1	0·0	4·5	4·4	7·4	19·1	8·5	3·9

London and Delhi results: mean of three tests. Teheran results: mean of two tests.
Values expressed to: whole numbers for valid monkeys, and first decimal places for virus doses and percentages.

* 'Spread': Cervical cord and brain levels with histological neuronal poliomyelitis.

TABLE 4. Summary results of intraspinal tests showing percentages of 'target' segments/regions/nuclei with neuronal lesions

	Poliovirus serotype								
	I Institutes			II Institutes			III Institutes		
	London	Delhi	Teheran	London	Delhi	Teheran	London	Delhi	Teheran
Maximum dose of virus injected (log_{10})	6·3	5·9	6·3	6·1	7·1	6·2	6·3	6·8	6·2
No. of monkeys with valid injection	25	29	28	27	28	27	26	30	30
Percentages of positive segments/ regions/nuclei of cervical cord and brain	1·5	0·4	0·0	1·0	1·0	2·5	7·8	2·1	1·5

London and Delhi results: Mean of three tests. Teheran results: Mean of two tests.
Values expressed to: whole numbers for valid monkeys, and first decimal places for virus doses and percentages.

TABLE 5. Summary results of the intracerebral tests expressed as numbers and percentages of monkeys with neuronal lesions in the thalamus and lumbar cord

		Poliovirus serotype								
		I Institutes			II Institutes			III Institutes		
		London	Delhi	Teheran	London	Delhi	Teheran	London	Delhi	Teheran
Virus dose injected (log_{10})		7·9	6·6	7·2	7·6	7·5	7·1	7·8	6·9	7·1
No. of valid monkeys		16	75	55	14	85	59	17	78	42
Histological neuronal poliomyelitis										
Thalamus:	No.	4	0	4	1	0	0	6	3	0
	%	25·0	0·0	7·3	7·1	0	0	35·3	3·8	0
Lumbar cord:	No.	1	3	0	0	2	5	2	2	0
	%	6·2	4·0	0·0	0·0	2·3	8·6	11·8	2·5	0

London and Delhi results: total of three tests.
Teheran results: Total of three tests for types I and II and a total of two tests for type III.
Values expressed to first decimal places for virus doses and percentages.

The overall i.c. results, indicating the relative sensitivity of the three species of monkey to the three poliovirus serotypes, are therefore similar to those obtained after i.s. inoculation.

DISCUSSION

The effect of the species of monkey in the performance and evaluation of the neurovirulence test has been considered by various workers (see Intro duction). The results of both the intraspinal and intracerebral neurovirulence tests in the three Institutes imply that the order of sensitivity of the monkey species to the Sabin vaccine strains, as judged by the presence of specific neuronal lesions in the central nervous system, is cynomolgus followed by rhesus, especially to the sero-types I and III attenuated poliovirus. Vervet monkeys appeared to be less sensitive than cynomolgus or rhesus monkeys to Sabin strains I and III, but appeared more sensitive than the other two species to type II. The lower sensitivity of vervet monkeys to types I and III poliovirus should not bias or preclude their use in the neurovirulence test, provided that the same species is employed for both the reference and test virus preparations.

The results from the three Institutes indicate the histological differences, following either intraspinal or intracerebral inoculation, produced-by the three serotypes of attenuated poliovirus with each species of monkey. The need for homotypic references when performing the neurovirulence-test is emphasized by the findings we report.

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