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The influence of technique on the sensitivity of monkeys to intraspinally inoculated poliovirus

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INTRODUCTION

Sabin (1959) has pointed out that the results of neurovirulence tests of strains of poliovirus by the intraspinal route depend in large measure on the technique of injection. The less virulent the strain being tested the more important does this factor become. On the other hand, with strains of high neurovirulence, such as the Mahoney strain of Type 1 poliovirus, it may be impossible to demonstrate any effect of technique on the result of the test, provided some, at least, of the inoculum is deposited in the cord.

Although it appeared at one time that the diameter of the needle was critical (Sabin, 1959), it seemed to us that the actual technique of inserting it might be of more consequence than its size. Sabin pushes the needle through the cord until he strikes the bone of the ventral wall of the spinal canal and then withdraws it 1–2 mm. He has shown that within these limits the further the needle is withdrawn the more sensitive is the test. Other workers insert the needle until the tip is within the cord as judged by a muscular twitch and then make the injection slowly.

We shall refer to this latter method as the 'standard technique' as it is the one we have always used in the testing of inactivated vaccines. When it is used there should be more or less continuous fibrillation of the muscles of one or both lower limbs throughout the injection. Melnick, Benyesh-Melnick & Brennan (1959) move the tip of the needle slightly during the injection.

Observations on specimens derived from safety-tests of inactivated vaccines led us to conclude that where a needle-track can be demonstrated passing right through the spinal cord there is but little extension of the inoculation trauma up and down the cord. By contrast, in the majority of specimens, the needle-track fails to reach the ventral surface of the cord, and evidence of inoculation trauma usually extends over several segments. It therefore seemed reasonable to suppose that the dispersal of inoculum deposited in the cord depends on the presence or absence of an artificially formed outlet to the ventral surface of the cord. When such an outlet exists, the bulk of the inoculum passes through it out of the cord into the subarachnoid space on its ventral surface. When such an outlet is not available, it does not burst through to the ventral surface of the cord, but tracks up and down in the grey matter and posterior columns. In order to elucidate this problem further, we undertook two experimental studies, one with indian ink as inoculum and the other with Sabin's Leon 12 a_1b strain of Type 3 poliovirus.

METHODS AND MATERIALS

Animals

For the indian ink experiments Rhesus monkeys (*Macaca mulatta*) weighing 1·5–2·5 kg, were used, and for the experiment with live virus cynomolgus monkeys (*Macaca irus philippinensis*) weighing between 1·4 and 1·8 kg. All the animals appeared healthy and had been in quarantine for at least 4 weeks. For inoculation they were anaesthetized by the intraperitoneal injection of pentobarbitone sodium.

Virus

The virus pool used had been prepared by subculturing Dr Sabin's original large lot of his Leon 12 a_1b Type 3 strain of poliovirus (Sabin, 1957) in monkey kidney monolayers maintained at 33° C. during virus multiplication. The titre was 2×10^8 TCID 50 per ml. Tenfold dilutions were prepared in Medium 199 (Morgan, Morton & Parker, 1950).

Inoculation

Each animal received 0·1 ml. of inoculum either by Sabin's technique or by the 'standard technique' through the space between the first and second lumbar vertebrae. In order to eliminate the influence of the size of the needle, all injections were made with a 24 s.w.g. needle with a short bevel. The actual injections were made slowly and, as far as possible, without moving the tip of the needle. We found some difficulty in assessing how far the needle had been withdrawn from the ventral surface of the spinal canal when using Sabin's technique, but, as far as we could judge, the needle was withdrawn 1–2 mm. before injecting the fluid.

Histological and clinical examinations

Indian ink experiments. A few minutes after the completion of the injection the spinal cords were removed together with the dura mater and fixed by immersion in 10% formol saline.

Virus experiment. The monkeys were examined daily for 21 days and symptoms recorded. Any animal showing definite weakness of one or more limbs, not attributable to injection trauma, is recorded as paralysed in the table. At the end of the observation period the survivors were anaesthetized and perfused with formol-saline (Biological Standards Control Laboratories (B.S.C.L.), 1957). Ten blocks were taken from each of the spinal cord enlargements, embedded in paraffin, sectioned at 15μ and stained with gallocyanin (Beswick, 1958).

RESULTS

Indian ink experiments

An immediate difference in the appearance of the cords was noted before the dura was opened or the specimens fixed; it was clear that there was more ink in the subarachnoid spaces of animals injected by the Sabin technique than in the subarachnoid spaces of animals injected by the 'standard technique'. However, even the latter contained enough ink to make it obvious that a substantial part of the

inoculum had either leaked back past the needle during the injection or regurgitated along the needle-track after the needle had been withdrawn. After fixation the membranes were stripped from the cords, which were then cleared by the Spalteholz technique. Pl. 1 shows a typical pair of such cleared specimens and the distribution of ink in the two cords affords an excellent demonstration of the different distribution of inoculum achieved by the two methods.

Experiment with live virus

Encouraged by the results of the preliminary experiments we carried out simultaneous titrations of Sabin's Type 3 strain in two groups of monkeys, one inoculated by his technique and one inoculated by the 'standard technique'.

Table 1. *The effect of technique of injection on the sensitivity of cynomolgus monkeys to attenuated poliovirus (Type 3) inoculated intraspinally*

(Each animal received 0·1 ml. of inoculum through the space L_1–L_2, using a 24 s.w.g. needle. (PP = partial paralysis of one or both lower limbs.))

Dilution and dose	No. of animals injected	No. developing paralysis	No. with polio lesions: Lumbar cord	No. with polio lesions: Cervical cord
	A. Sabin's method of injection			
10^0 (2×10^7 TCID 50)	8	0	3	2
10^{-1} (2×10^6 TCID 50)	7	2	4	4
10^{-2} (2×10^5 TCID 50)	7	3	3	3
10^{-3} (2×10^4 TCID 50)	8	0	2	1
	B. Standard method of injection			
10^0 (2×10^7 TCID 50)	8	8 (3 PP)	8	8
10^{-1} (2×10^6 TCID 50)	8	4 (1 PP)	7	3
10^{-2} (2×10^5 TCID 50)	8	4	8	5
10^{-3} (2×10^4 TCID 50)	8	0	4	3
Totals paralysed	A. 5/30 (17 %) $\chi^2 = 6{\cdot}26$ P = approx. 0·013		B. 16/32 (50 %)	
Totals with poliomyelitis lesions	A. 12/30 (40 %) $\chi^2 = 13{\cdot}26$ $P = < 0{\cdot}001$		B. 28/32 (87 %)	
Average no. of levels per monkey showing inoculation trauma	A. 1·6/10		B. 4·0/10	

The results of this experiment are summarized in Table 1. The incidence of paralysis and the incidence of poliomyelitis lesions are both significantly higher in the group injected by the 'standard' method. Even if animals showing neither inoculation trauma in the grey matter nor poliomyelitis lesions are excluded the differences between the two groups remain significant (P = approx. 0·007 for poliomyelitis lesions and P = approx. 0·02 for paralysis). The average numbers

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of levels showing inoculation trauma in the two groups emphasize the wider initial distribution of the inoculum in the group injected by the 'standard' method.

There was no significant difference in the severity or extent of the poliomyelitis lesions in the affected monkeys of the two groups.

DISCUSSION

It is clear that Sabin's method of intraspinal injection is less sensitive than the 'standard' method. However, in deciding which of the two methods to adopt, the purpose for which it is to be used must be taken into consideration. Obviously, when maximum sensitivity is the only criterion, as in the safety testing of inactivated poliomyelitis vaccines, the Sabin technique is inferior. Indeed, the practice of this laboratory, which is to give three separate injections in adjacent intervertebral spaces (T_{12}–L_1, L_1–L_2 and L_2–L_3) when testing inactivated vaccine (B.S.C.L. 1957) probably affords an even more sensitive technique than the single injection in use in most testing laboratories.

On the other hand, when testing strains of poliovirus for intraspinal neurovirulence, sensitivity is not usually the only consideration. Reproducibility and ease of interpretation are equally important. Sabin's method produces less traumatic paralysis, making clinical assessment of the animals easier. As it usually, although in our experience not always, places the inoculum on one side of the cord in one or two segments only, it is easier to distinguish active spread of the virus across or up and down the cord from passive spread produced by wide initial dispersal of the inoculum. We have insufficient data to assess the relative reproducibility of the two methods. The published evidence suggests that Sabin's method gives results which are at least as reproducible as other methods. It is probable that any method of intraspinal injection can be made the basis of neurovirulence tests on attenuated strains provided that at least some of the inoculum is placed in the grey matter of the lumbar cord and that, in the hands of the person using it, it gives consistent results in terms of placement and initial distribution of the inoculum. What cannot be done is to compare results obtained by one technique directly with results obtained by another. Small details of technique are so important that it is probably unwise to assume that monkeys injected by different operators are comparable, even though both claim to use the same method.

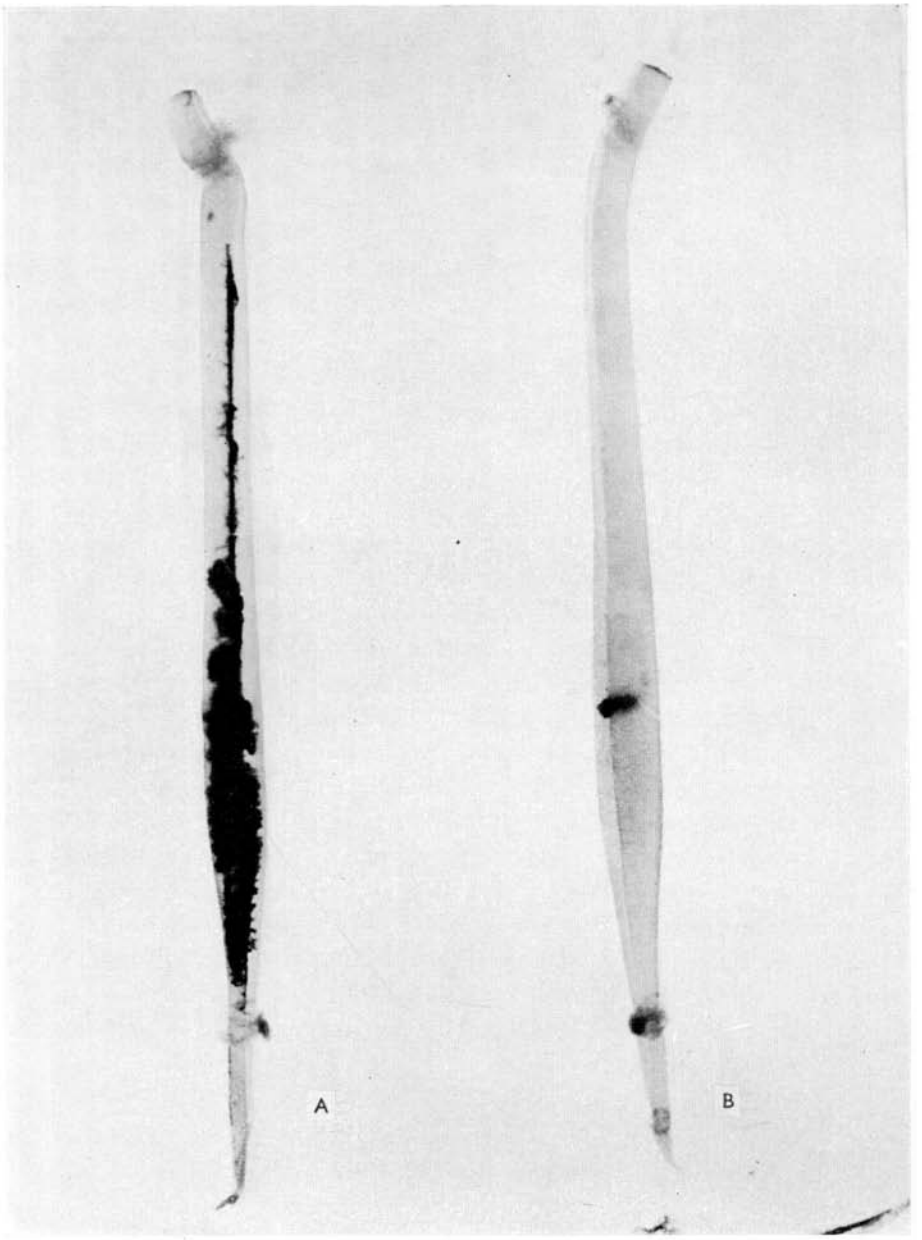
SUMMARY

1. Two methods of injecting monkeys intraspinally are compared.

2. It has been shown that the technique of inserting the needle through the cord and withdrawing it 1–2 mm. before injection (Sabin's technique) gives a less widespread distribution of inoculum than the alternative method of making the injection as soon as the tip of the needle is within the substance of the spinal cord ('standard technique').

3. When attenuated Type 3 poliovirus is injected into cynomolgus monkeys by Sabin's technique, the incidence of paralysis and poliomyelitis lesions observed is less than when it is injected by the 'standard technique'.

4. The relative merits of the two techniques are discussed.



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EXPLANATION OF PLATE

The caudal halves of the spinal cords of monkeys inoculated with 0·1 ml. of indian ink. A. By the 'standard technique'. B. By Sabin's technique. Specimens cleared by the Spalteholtz method. (× 2 natural size.)