The behaviour of attenuated strains of poliovirus in monkeys

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The classical descriptions of the character and distribution of the lesions in experimental poliomyelitis in the monkey nearly all relate to animals inoculated with virulent strains of virus capable of producing paralysis after intracerebral inoculation (see, for example, Hurst, 1929; Bodian, 1948, 1959; Bodian and Howe, 1940). No such detailed accounts of the pathological changes in animals infected with highly attenuated strains of virus, like those currently in use as oral poliomyelitis vaccines, have so far appeared in the literature. Pette, Lennartz, Maass, Valenciano, and Mannweiler (1960) discuss the 'pathological anatomy' very briefly, saying that the changes 'corresponded to those seen in not too severe poliomyelitis'. Khesin, Gendon, Levenbuk, and Rozina (1961) suggest that the differences between virulent and attenuated strains are sufficiently reproducible to be made the basis of a morphological marker which they suggest should be called the M-character. Unfortunately, they do not give precise criteria for its determination. In this paper the character, extent, and distribution of the lesions produced in monkeys by attenuated strains are described and compared with those seen in animals infected with more virulent strains and an attempt is made to assess the feasibility of defining one or more marker characters based on the differences between the two.

MATERIALS AND METHODS

The descriptions of the changes induced by attenuated strains of poliovirus are based on the examination of some 2,000 monkeys inoculated intramuscularly, subcutaneously, intraspinally, or intracerebrally with either Sabin's strains (LSc 2 ab, type 1; P-712 Ch. 2 ab, type 2, and Leon 12 a,b, type 3) or the attenuated strains of Cox or Koprowski. Both cynomolgus (Macaca irus) and rhesus (M. mulatta) monkeys have been used. The brains and spinal cords of rhesus and cynomolgus monkeys have also been examined after inoculation by various routes of the Bruneders strain of type 1 virus (Enders, Weller, and Robbins, 1952; Sabin, 1955; Gear, 1956; Biological Standards Control Laboratory, 1957) MEF1 (type 2), Saukett (type 3), Mahoney (type 1) as well as various wild strains of all three types. The sections from rhesus and cynomolgus monkeys employed in the testing of inactivated vaccines have given valuable data on the types of lesion likely to occur spontaneously or as the result of inoculation trauma.

HOUSING AND CARE OF MONKEYS The methods of care and housing have already been described (Coid, 1959).

TECHNIQUES OF INOCULATION Animals inoculated intracerebrally received either 0-5 ml or 0-3 ml into each thalamus through a burr hole on or close to the coronal suture and 0-5 cm. from the midline. A metal guide was employed to place the burr holes and a 27 s.w.g. needle, 1½ in. long was used for the injections. Intraspinal injections of 0-1 ml were given through the intervertebral space L7-L8 or, in some cases, T12-L1, L4-L5, or all three of these spaces. A 1 in., 24 s.w.g. needle was employed and injections were made either by Sabin's method (Sabin, 1959) or more frequently by the 'standard technique' of Beswick and Coid (1961). Intramuscular injections were usually into the deltoid muscle and subcutaneous injections under the skin of the chest wall about 2 cm. lateral to the nipple (Coid, Beswick, and Tobin, 1961).

HISTOLOGICAL METHODS All but a few of the animals were fixed by perfusion with 10% formal saline (4% HCHO) containing 3% v/v of glacial acetic acid (Biological Standards Control Laboratory, 1957; Bywater and Glees, 1959). After embedding in paraffin, sections were cut at 15μ and stained by a modification of Einarson's galloycyanin-lake technique (Beswick, 1958). The levels examined were usually those described by Coid, Beswick, and Tobin (1961) except that 14 blocks (L4-S5 inclusive) were taken from all intraspinally inoculated animals (Beswick and Winter, 1961).

HISTOLOGICAL FINDINGS

Except where the contrary is specifically stated, reference to the lesions produced by attenuated
strains of virus refers to those seen in cynomolgus monkeys inoculated intraspinally with one or other of Sabin's vaccine strains. The large number of intracerebrally inoculated animals has contributed less, as all but a few showed either no histological evidence of infection or only limited evidence of virus activity restricted to the immediate vicinity of the inoculation site. In general, Cox's and Koprowski's strains have behaved like Sabin's except for a greater tendency to spread from the inoculation site to distant parts of the central nervous system and a tendency to produce somewhat more severe lesions. Much smaller numbers of animals were inoculated subcutaneously and intramuscularly. Consequently, detailed analysis of the results of infection in these routes has not been attempted.

SEVERITY OF LESIONS The only basis for assessing the severity of poliomyelitis lesions which is not subject to intolerable error is the proportion of large neurones destroyed. To determine this with any precision requires the cutting of serial sections of the diseased and comparable normal tissue. However, an experienced observer can make a reliable, if rather crude, estimate from the examination of a number of single sections (Beswick and Winter, 1961). Using this method of assessment, it is clear that lesions due to attenuated strains are in general less severe than those produced by more virulent strains. This is particularly true of lesions remote from the inoculation site and is no more than would be suspected from the greater tendency of virulent strains to cause paralysis. Khesin et al. (1961) apparently made this difference the principal basis for the determination of their M-character. However, the variation in the severity of lesions in different monkeys receiving the same inoculum is so great that unless very large numbers of animals were used, only gross differences in neurovirulence could be detected with certainty. It is probably most satisfactory, if an assessment on this basis is to be attempted, to follow Sabin (1959) and make the presence or absence of paralysis the criterion of severity. However, even when this method is adopted, much depends on the technique of inoculation used (Beswick and Coid, 1961).

Although there is a great deal of overlap, lesions due to Sabin's viruses tend to be less severe in cynomolgus monkeys inoculated low in the lumbar cord than in those inoculated higher up, and in normal animals than in those receiving large doses of cortisone. Rhesus monkeys are less susceptible than cynomolgus monkeys to attenuated strains of virus, including the partially attenuated Brunenders strain of type 1, and the lesions tend to be less severe. Vervet (Cercopithecus) and Huzzar (Patas) monkeys appear to resemble rhesus rather than cynomolgus monkeys. Of the different routes of inoculation studied, the most severe lesions follow intraspinal injection with all the virus strains tested. The least sensitive route is the subcutaneous, but the lesions, when they do occur, may be more severe and extensive than those following intracerebral inoculation. With type 1 viruses (Cox and Sabin) intramuscular injection gave rise to more severe lesions than intracerebral inoculation (Coid et al., 1961). Types 2 and 3 have been insufficiently studied by intramuscular inoculation to say whether this route is the more sensitive for these types too.

THE EXTENT OF LESIONS Various workers (Cabasso, Jungherr, Levine, Moyer, Roca-Garcia, and Cox, 1960; Jungherr, 1960) have suggested that the capacity of a virus to spread long distances from the site of inoculation in cord or brain is a measure of its neurovirulence. It is certainly true to say that lesions remote from the inoculation site are more constant in animals infected by virulent strains; however, such lesions frequently occur in animals inoculated with attenuated strains (Tables I and II), provided a proper search is made for them. Assessments of neurovirulence based on the distance separating the inoculation site from the most remote lesions are entirely misleading. Attenuation of polioviruses results, not in any striking reduction in their capacity to travel long distances in the central nervous system, but in an increased tendency for them to do so without leaving any evidence of their passage and without much local spread when they reach their destination. Table II shows that after intracerebral inoculation of type 1 the lumbar cord is affected more often than the cervical cord or thoracic cord; indeed, lesions in the lumbar cord often constitute the only evidence of virus activity outside the immediate neighbourhood of the inoculation site. On the other hand, inoculation into the lumbar cord often produces solitary lesions in the red nucleus, lateral vestibular nucleus, or reticular formation of the medulla with no evidence of virus activity in thoracic or cervical cords. The restricted capacity of attenuated virus to spread locally is well shown in Fig. 1, in which part of one anterior horn has been destroyed and the rest of the grey matter spared. With more virulent viruses such sharply limited lesions are seldom if ever observed. A lesion of this severity in one anterior horn would be associated at least with involvement of the opposite anterior horn and usually with lesions in the posterior and central grey matter as well. It is this striking capacity of the attenuated viruses to produce clearly defined lesions which has made possible the detailed
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analysis of the distribution of lesions described below and shown in Tables I and II.

DISTRIBUTION OF THE LESIONS  Even the earliest students of the pathology of acute poliomyelitis were aware that the lesions were not distributed randomly in the central nervous system (Wickman, 1907; Peabody, Draper, and Dochez, 1912). However, in spite of the observations of these authors and of others who have studied experimental

| TABLE I |
| DISTRIBUTION OF POLIOMYELITIS LESIONS IN MONKEYS INOCULATED INTRASPINALLY |
| | Type 1 | Type 2 | Type 3 | All Types Combined |
| | No. Positive | % Positive | No. Positive | % Positive | No. Positive | % Positive | No. Positive | % Positive |
| Spinal cord | | | | | | | | |
| Lumbar | 161 | 98 | 138 | 99 | 160 | 94 | 459 | 97 |
| Thoracic | 77 | 47 | 47 | 34 | 96 | 57 | 220 | 47 |
| Cervical | 77 | 47 | 71 | 51 | 97 | 57 | 245 | 52 |
| Brain-stem nuclei (non-motor) | | | | | | | | |
| Lat. reticular | 24 | 18 | 17 | 12 | 46 | 27 | 87 | 18 |
| Lat. cuneate | 13 | 8 | 7 | 5 | 30 | 18 | 50 | 11 |
| N. gracilis and cuneatus | 1 | 1 | 0 | 0 | 5 | 3 | 6 | 1 |
| Infr. olivary | 14 | 9 | 9 | 3 | 24 | 14 | 42 | 9 |
| Central reticular formation of medulla | 63 | 38 | 61 | 44 | 67 | 40 | 191 | 41 |
| N. of spinal Tr. of V | 2 | 1 | 1 | 1 | 6 | 4 | 9 | 2 |
| Lat. vestibular | 56 | 34 | 33 | 22 | 54 | 32 | 143 | 30 |
| Other vestibular | 6 | 4 | 3 | 2 | 24 | 14 | 33 | 7 |
| Cochlear | 1 | 1 | 0 | 0 | 8 | 5 | 9 | 2 |
| Dentate | 13 | 8 | 12 | 9 | 36 | 21 | 61 | 13 |
| Emboliform/globose | 16 | 10 | 10 | 7 | 42 | 25 | 68 | 14 |
| Fastigial | 12 | 7 | 8 | 6 | 32 | 19 | 52 | 11 |
| Red nucleus | 85 | 52 | 69 | 50 | 68 | 40 | 222 | 47 |
| Substantia nigra | 6 | 4 | 8 | 6 | 32 | 19 | 46 | 10 |
| Tegmentum | 2 | 1 | 6 | 4 | 24 | 14 | 32 | 7 |
| Tectum and pretectum | 1 | 1 | 1 | 1 | 13 | 8 | 15 | 3 |
| Nn. pontis | 0 | 0 | 3 | 2 | 10 | 6 | 13 | 3 |
| Periaqueductal grey matter | 2 | 1 | 1 | 1 | 21 | 12 | 24 | 5 |
| Intestitital | 1 | 1 | 2 | 1 | 9 | 5 | 12 | 3 |
| N. of Darkschewitsch | 2 | 1 | 2 | 1 | 8 | 5 | 12 | 3 |
| Cranial motor nuclei | | | | | | | | |
| III | 2 | 1 | 3 | 2 | 18 | 11 | 23 | 5 |
| IV | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 0-4 |
| VI | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 0-4 |
| VII | 1 | 1 | 2 | 1 | 6 | 4 | 9 | 2 |
| N. ambiguous | 2 | 1 | 5 | 4 | 4 | 2 | 11 | 2 |
| Dorsal motor n. of vagus | 1 | 1 | 1 | 1 | 6 | 4 | 8 | 2 |
| XII | 0 | 0 | 0 | 0 | 9 | 5 | 9 | 2 |
| Forebrain structures | | | | | | | | |
| Medial geniculate body | 0 | 0 | 0 | 0 | 4 | 2 | 4 | 1 |
| Thalamus—lateral | 12 | 7 | 4 | 3 | 30 | 18 | 46 | 10 |
| Thalamus—medial and anterior | 2 | 1 | 2 | 1 | 13 | 8 | 17 | 4 |
| Corpus Luysii (subthalamic N.) | 1 | 1 | 3 | 2 | 20 | 12 | 24 | 5 |
| Zona incerta | 2 | 1 | 2 | 1 | 8 | 5 | 12 | 3 |
| Hypothalamus | 4 | 2 | 2 | 1 | 8 | 5 | 14 | 3 |
| Globus pallidus—medial | 1 | 1 | 3 | 2 | 15 | 9 | 19 | 4 |
| Globus pallidus—lateral | 0 | 0 | 0 | 0 | 2 | 1 | 2 | 0-4 |
| Putamen | 0 | 0 | 1 | 1 | 2 | 1 | 3 | 1 |
| Precentral gyrus | 13 | 8 | 14 | 10 | 29 | 17 | 56 | 12 |
| Postcentral gyrus | 1 | 1 | 0 | 0 | 12 | 7 | 13 | 3 |
| Paracentral lobule | 0 | 0 | 0 | 0 | 4 | 2 | 4 | 1 |
| Other parietal lobe cortex | 0 | 0 | 2 | 1 | 5 | 3 | 7 | 1 |
| Temporal lobe cortex | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 1 |
| Cortex of insula | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 1 |
| Rhiemencephalon | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0-2 |
| Cerebellar cortex | 1 | 1 | 0 | 0 | 4 | 2 | 5 | 0-4 |

1 Cranial motor nuclei of IV, V, and VI not examined in all cases, therefore true values may be higher than those shown, especially for type 3. However, the figures are of the right order of magnitude.

Percentages given to nearest whole number except values under 0.5% which are given to nearest 0.1%.
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TABLE II

DISTRIBUTION OF Lesions IN MONKEYS INOCULATED INTRACEREBRALLY

<table>
<thead>
<tr>
<th>Total Monkeys Positive</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>All Types Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td></td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Spinal cord</td>
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<td></td>
</tr>
<tr>
<td>Lumbar</td>
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<td>14</td>
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<td>9</td>
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<td>0</td>
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<td>Cervical</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>11</td>
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<tr>
<td>Brain-stem nuclei (non-motor)</td>
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<td>Lat. reticular</td>
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<td>9</td>
<td>1</td>
<td>3</td>
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<td>7</td>
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<td>0</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>7</td>
<td>1</td>
<td>3</td>
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<td>10</td>
<td>7</td>
<td>19</td>
</tr>
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<td>N. of spinal Tr. of V</td>
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<td>0</td>
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<td>7</td>
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<td>3</td>
<td>0</td>
<td>0</td>
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<td>Cochlear</td>
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<td>0</td>
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<tr>
<td>Dentate</td>
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<td>5</td>
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<td>Emboliform/globus</td>
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<td>7</td>
<td>1</td>
<td>3</td>
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<td>Fastigial</td>
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<td>1</td>
<td>3</td>
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<tr>
<td>Red nucleus</td>
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<td>17</td>
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<td>3</td>
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<td>11</td>
<td>19</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Tectum and pretectum</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Cranial motor nuclei</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
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<td>VII</td>
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<td>0</td>
</tr>
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<td>0</td>
</tr>
<tr>
<td>Forebrain structures</td>
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<td></td>
<td></td>
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<td>Medial geniculate body</td>
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<td>3</td>
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<td>0</td>
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<td>Thalamus</td>
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<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Globus pallidus</td>
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<td>7</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Putamen</td>
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<td>0</td>
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<td>Precentral gyrus</td>
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<td>Postcentral gyrus</td>
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<td>2</td>
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</tr>
</tbody>
</table>

1Figures in parenthesis are nos. of animals with lesions restricted to the immediate vicinity of the inoculation site. Such animals have not been taken into account in calculating the percentages in the table.

2Virus activity close to inoculation site(s)—not evidence of spread.

3When lesions occur in these structures it is usually difficult to decide to what extent active spread has occurred from the inoculation site since they are liable to be involved in inoculation trauma.

FIG. 1. Cynomolgus monkey inoculated intraspinally with approximately 1,000 TCID<sub>50</sub> of Sabin's type 2 virus, P 712, Ch, 2 ab, and killed 21 days later; no paralysis. Section of mid-lumbar cord to show a sharply circumscribed lesion affecting part of one anterior horn only. Note absence of lesions in opposite anterior horn and both posterior horns. Gallocyanin. × 10.
poliomyelitis in the monkey (Hurst, 1929; Bodian and Howe, 1947; Bodian, 1949) there has been a tendency in many quarters to oversimplify the picture by laying too much emphasis on the importance of the anterior horn cells of the spinal cord and of their cranial homologues. Involvement of other types of cell has been regarded as almost accidental, representing an overflow of the virus from the sites of primary multiplication into neighbouring areas of lesser susceptibility. If this view were correct it would be expected that, as viruses of progressively lower neurovirulence were studied, these 'overflow' lesions would be seen less and less until a point was reached where lesions were entirely restricted to the anterior horns of the spinal cord and those cranial nerve nuclei which contain homologous neurones. If Sabin's strains are injected into the lumbar cord, animals which show histological evidence of infection nearly, but not quite, always show lesions in the anterior horn of the spinal cord at one level at least. However, the lesions in the posterior horns are sometimes more severe than those in the anterior horns and individual levels may be encountered in which there is severe neuronal destruction in, for example, Clarke's column with no evidence of involvement of the anterior horns at the same level (Fig. 2). Such a finding is rarely, if ever, made with virulent strains (Bodian, 1948). After intracerebral inoculation it is by no means unusual to encounter lesions in the lumbar cord which are restricted to the posterior horn. There can be little doubt about the specificity of the majority of such lesions; not only is their appearance consistent with their being due to poliovirus, but they have not been encountered in the large number of monkeys injected with inactivated poliomyelitis virus. Attenuated viruses seem to pass more readily up and down the cord than across it. When the inoculation site is clearly confined to one side, signs of virus activity in the lumbar cord are often almost entirely restricted to the same side.

Lesions in the cranial homologues of the anterior horns of the cord are the exception in animals inoculated intraspinally, intracerebrally, subcutaneously, or intramuscularly with Sabin's strains, types 1 and 2. Sabin's type 3 strain produces such lesions more frequently, but as can be seen from Tables I and II they are still not common. Perhaps the most striking feature of Tables I and II is the evidence they afford of the capacity of even highly attenuated viruses to reach remote parts of the central nervous system. Following intraspinal inoculation, brain lesions are seen in 66% of animals, provided a thorough search is made and provided the quality of the histological preparations is adequate to permit the recognition of minor changes and their specificity to be established. This figure is higher than most published figures (Murray, Kirschstein, Van Hoosier, and Baron, 1959). The lower rate of spread to the brain observed by these authors (about 30% for Sabin's strains) is probably due to the fact that they used only rhesus monkeys.

The technique of intraspinal injection is also of importance in determining the tendency of the viruses to spread (Beswick and Coid, 1961). However, brain lesions are not randomly distributed and are usually restricted to a comparatively few cell groups. After intraspinal inoculation, brain lesions are most frequently observed in the red nucleus (magnocellular portion) (Fig. 3), the lateral vestibular nucleus (Deiter's nucleus) (Fig. 4), and the central reticular formation of the medulla, especially the large, so-called 'motor cells'. Rather less often affected (10 to 20%) are the lateral reticular nucleus; the dentate, emboliform, globose, and fastigial nuclei in the central grey matter of the cerebellum; the lateral cuneate nucleus; the inferior olivary nucleus; the lateral nucleus of the thalamus; the substantia nigra and the motor cortex. Although exact figures are not available for all of them it is clear that the cranial motor nuclei are not sites of election of virus multiplication. Table I shows that there are significant differences between the three types of virus in respect of the distribution of lesions produced after intraspinal inoculation. The most striking differences are between type 3 and the other two types. The type 3 virus has a greater tendency to produce lesions in the substantia nigra, tegmentum, periaqueductal grey matter, subthalamic nuclei, and medial vestibular nuclei. Type 3 has also a greater, though not very marked, tendency to cause lesions in cranial motor nuclei and in the sensory cortex (postcentral gyrus). The differences between types 1 and 2 are less striking. Types 1 and 3 viruses produce lesions in the thoracic cord as often as in the cervical cord, whereas type 2 produces cervical cord lesions one-and-a-half times as often as thoracic cord lesions. These differences are significant at the 1% level ($\chi^2$ test) and the difference between types 1 and 2 in the frequency of lesions in the lateral vestibular nucleus is significant at the 5% level.

Of particular interest from the point of view of elucidating the pathways whereby virus spreads in the central nervous system are the sites of what may be called solitary brain lesions, that is, lesions restricted to a single structure or pair of structures. Such lesions are only common in three situations: the magnocellular part of the red nucleus, the lateral vestibular nucleus, and the central reticular formation of the medulla. They also occur, but much less commonly, in the lateral reticular nucleus, lateral cuneate nucleus, and substantia nigra (type 3 only). A few animals have shown solitary lesions
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FIG. 2

Cynomolgus monkey inoculated intraspinally with approximately $10^{4.5} \text{TCID}_{50}$ of Sabin's type 1 virus, LSc, 2 ab, and killed 22 days later. No paralysis. Section of upper lumbar cord to show destruction of Clarke's column with no evidence of virus activity in the anterior horns. Gallocyanin. $\times 10$.

FIG. 3

Cynomolgus monkey inoculated intraspinally with approximately $10^{4.0} \text{TCID}_{50}$ of Sabin's type 2 virus, P 712, Ch, 2 ab, and killed 21 days later. No paralysis. Almost total destruction of the right red nucleus with minimal involvement of the left. The lesion on each side is restricted to the magnocellular portion of the nucleus. Gallocyanin. $\times 5$.

FIG. 4

Cynomolgus monkey inoculated intraspinally with approximately $10^6 \text{TCID}_{50}$ of Sabin's type 3 virus, Leon 12, a,b, and killed 22 days later. No paralysis. Virus activity in left lateral vestibular nucleus. The lateral angle of the fourth ventricle is at the top right hand corner of the photograph. Gallocyanin. $\times 50$. 
FIG. 5. Cynomolgus monkey inoculated intraspinaly with approximately $10^{3.5}$ TCID$_{50}$ of Sabin's type 1 virus, LSc, 2 ab, and killed 22 days later. Partial paralysis of left leg. Note pericapillary round-cell infiltration and the absence of polymorphonuclear leucocytes and nuclear debris. Lumbar cord. Gallocyanin. $\times$ 730.

FIG. 6. Cynomolgus monkey inoculated intraspinally with approximately $10^{6}$ TCID$_{50}$ of Sabin's type 2 virus, P 712, Ch, 2 ab and killed 22 days later. Partial paralysis of right leg with onset four days after inoculation. The photograph shows a small part of the lesion shown at lower magnification in Fig. 7. The endothelial cells lining the capillaries are clearly shown with the surrounding mantles of inflammatory cells. Lumbar cord. Gallocyanin. $\times$ 730.
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which are probably specific at the rostral end of one inferior olivary nucleus. A single animal exhibited a lesion in one facial nucleus (VII), the only example of a solitary lesion in a cranial motor nucleus.

Lesions in the fastigial nucleus only occur when there are lesions in the homolateral (rarely contralateral) lateral vestibular nucleus. Lesions in the emboliform, globose, and dentate nuclei occur in association with lesions in the red nucleus of the opposite side. When involvement of one red nucleus is associated with lesions in one lateral vestibular nucleus, it is nearly always the nuclei of opposite sides which are affected. With a good many exceptions, unilateral lesions caudal to the anterior extremity of the fourth ventricle tend to be on the same side as the spinal inoculation trauma and the principal poliomyelitis lesions in the spinal cord. Unilateral lesions rostral to the fourth ventricle tend to be on the opposite side. No doubt, detailed analysis of additional data would reveal other combinations of lesions which occur more often than can be accounted for by chance.

The figures given in Table II are based on smaller numbers of positive monkeys but a number of points emerge, of which the most striking is perhaps the relatively higher incidence of lesions confined to the immediate vicinity of the inoculation site (42%) compared with intraspinally inoculated animals (34%), and the appearance of the substantia nigra as the principal site for brain lesions away from the inoculation site, eclipsing even the red nucleus, lateral vestibular nucleus, and central reticular formation of the medulla, although the reticular formation of the tegmentum is affected in 21% of histologically positive animals. Intertypic differences are not as conspicuous as they are in intraspinally inoculated monkeys. However, type 1 seems to show a slightly greater tendency to produce cord lesions and type 2 shows the least tendency to produce lesions remote from the inoculation site. Type 3 seems to affect the roof nuclei of the cerebellum more than either of the other types. No comparable tables showing the distribution of lesions in animals inoculated with virulent strains have appeared in the literature.

FIG. 7. The same as Fig. 6. The photograph shows a typical 'granulomatous type' lesion in the lumbar cord and displays clearly the striking appearance produced by the interlacing cords of inflammatory cells which ensheathe the capillaries as well as the larger vessels. This type of lesion, also shown in Figs. 1, 2, and 3, is characteristic of highly attenuated poliovirus but is rarely produced by more virulent strains. Contrast with Fig. 8. Galloccyanin. × 81.
However, a table such as that of Bodian (1947) shows the distribution of lesions in fatal human cases which is like that seen in monkeys infected with virulent strains. The tables may also be compared with Bodian's diagram (Bodian, 1959, Fig. 69).

**CHARACTER OF THE LESIONS**

The most comprehensive and detailed account of the character of the lesions of experimental poliomyelitis is that of Bodian (1948). However, this, in common with all earlier accounts of the pathological changes in acute experimental or natural poliomyelitis from the time of Rissler (1888), has been concerned chiefly with virulent strains of virus, usually causing paralysis and therefore of considerably greater neurovirulence than the strains being considered. Anyone who studies material from monkeys inoculated with strains of poliovirus of differing neurovirulence must become aware that there are striking differences of character between the lesions produced by virulent viruses and those produced by the attenuated strains. He will also soon realize that such differences can only be made the basis for distinguishing one virus strain from another if a number of animals are examined. Occasionally, even highly attenuated strains may, in an individual animal, give rise to lesions which are indistinguishable from those produced by much more virulent strains. It is less common for the opposite to happen, but it can do so with any but the most virulent strains of virus. The differences are what may be termed differences in modal behaviour and any one strain may produce varying pathological pictures in different animals receiving the same inoculum. Nevertheless, the lesions produced by attenuated strains of virus differ from those produced by more virulent viruses in a number of respects, other than their severity, extent, and distribution, which have already been discussed. Table III lists the main respects in which the lesions due to attenuated viruses differ from those due to virulent strains. It is clear from that table that not only do the lesions induced by attenuated strains lack some of the features of the lesions due to virulent strains of virus, but they possess certain features of their own. The various characters listed require some explanation. The first two items are really different aspects of the same thing. In lesions due to virulent viruses, polymorphonuclear leucocytes are abundant during the first few days; thereafter they diminish rapidly. However, they do not migrate from the tissue, they die in situ and their disintegrating nuclei form the haematoxyphil dust-like debris so characteristic of severe forms of poliomyelitis (Bodian, 1948). Polymorphonuclear leucocytes are not entirely absent from the lesions induced by attenuated strains of virus but they are much less numerous, being generally restricted to the immediate vicinity of cells undergoing neuronophagia, and almost absent from the intervening nervous tissue and the perivascular spaces. When neuronophagia is complete, the few polymorphonuclear leucocytes soon disappear leaving little or no nuclear debris behind them.

**TABLE III**

**FEATURES DISTINGUISHING LESIONS DUE TO VIRULENT AND ATTENUATED STRAINS OF POLIOMYELITIS VIRUSES**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Virulent</th>
<th>Attenuated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Polymorphonuclear leucocytes</td>
<td>++ +</td>
<td>+</td>
</tr>
<tr>
<td>2 Basophilic nuclear debris</td>
<td>++ +</td>
<td>+</td>
</tr>
<tr>
<td>3 Chromatolysis of surviving neurones</td>
<td>+ + +</td>
<td>+ (exc. type 3 + +)</td>
</tr>
<tr>
<td>4 Capillary haemorrhage</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5 Oedema</td>
<td>+ +</td>
<td>-</td>
</tr>
<tr>
<td>6 Meningeal infiltration (round cell)</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>7 Lesions in intact white matter</td>
<td>+ +</td>
<td>±</td>
</tr>
<tr>
<td>8 Pericapillary round-cell infiltration</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>9 Granulomatous lesions</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10 Age difference between lumbar and cervical cord lesions</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11 Early round-cell perivascular cuffing</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>12 Sharply localized lesions</td>
<td>-</td>
<td>+ + +</td>
</tr>
<tr>
<td>13 Severe involvement of posterior horns</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>without lesions in anterior horn at some level</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Chromatolysis of neurones which are not immediately destroyed by the virus is probably the most characteristic feature of acute poliomyelitis as ordinarily described (Bodian, 1948), but the virtual absence of more than minimal changes in surviving neurones is usually a striking feature of lesions due to Sabin's strains of type 1 and 2. Sabin's type 3 virus does produce some chromatolysis in most animals but even this is less than is usually seen with more virulent strains. Figures 9 and 10 show this difference between virulent and attenuated strains of virus.

Capillary haemorrhages and oedema may be considered together as manifestations of severe acute tissue damage. Both occur in the early stages of lesions and are obvious only when severe. Mild oedema of nervous tissue is difficult to recognize histologically; but with virulent strains of virus it is often sufficient to distort the outline of the grey matter and to produce obvious thinning of the glial and neuronal meshwork as shown by the appearance of pale-staining areas within the grey matter. Once the acute stage has passed oedema of course subsides, although evidence of small capillary haemorrhages may persist for a considerable time.

Round-cell infiltration of the meninges is seldom striking in the monkey, but is usually even less in animals infected with attenuated strains than in those inoculated with fully virulent viruses.

The relative absence of polymorphonuclear leucocytes from the lesions produced by attenuated
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FIG. 8. Javanese Cynomolgus monkey inoculated with a suspension of Brunender's virus containing approximately 40 TCID₅₀/ml as follows:
L. thalamus 0.5 ml.
Lumbar cord 0.25 ml. Total dose 80 TCID₅₀.
R. deltoid m. 1.25 ml.
The animal was 'conditioned' by the use of whole body x-irradiation and cortisone. Onset of paralysis (legs) six days after inoculation; killed 20 days after inoculation. The photograph shows the relatively scanty inflammatory infiltration in the anterior horn at a lumbar cord level from which the majority of the large motor neurones have disappeared. Even two weeks after the onset of paralysis, the appearance of this 'virulent type' lesion is in striking contrast to that of the 'granulomatous type' lesions of comparable age shown in Figs. 1, 2, 3, and 7. Gallocyanin. × 32.

FIG. 9. The same specimen as in Fig. 8. The photograph shows two of the surviving anterior horn cells, both showing severe chromatolysis, with early signs of recovery (Kern-kappe). The appearance of these cells corresponds exactly to one of the stages described in the 'classical' accounts of poliomyelitis (Bodian, 1948). Contrast with Fig. 10. Gallocyanin. × 260.
**FIG. 10.** Cynomolgus monkey inoculated intraspinally with approximately $10^{4.5}$ TCID$_{50}$ of Sabin's type 2 virus P 712, Ch, 2 ab, and killed 21 days later. Slight weakness of right leg from day +3 to day +15. Section of lumbar cord showing substantially normal anterior horn cells in an area of heavy cellular infiltration at a level from which the great majority of the large motor neurones have been lost. Contrast with Fig. 9. Gallocyanin. $\times$ 240.

**FIG. 11.** Cynomolgus monkey inoculated intraspinally with approximately $10^{4.5}$ TCID$_{50}$ of Sabin's type 2 virus P 712, Ch, 2 ab, and killed 21 days later. Weakness in legs developed eight days after inoculation and progressed slowly. Section to show intense virus activity in damaged white matter of lumbar cord. Contrast with Fig. 12. Gallocyanin. $\times$ 50.
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viruses is associated with the early appearance of large numbers of mononuclear cells both in the tissue and in the perivascular spaces. Massive perivascular cuffing and dense cellular infiltration of grey matter may be seen in any animal which has severe lesions and survives long enough. If the lesions are more than two or three weeks old there may be little difference in this respect between lesions produced by strains of widely different neurovirulence. However, if lesions are examined earlier, those due to attenuated viruses nearly always show substantially more round-cell infiltration than lesions of comparable age and severity (in terms of neuronal destruction) due to virulent viruses (see Figs. 1, 2, 7, and 8). A characteristic feature of the lesions due to attenuated viruses is the presence of what has been termed in Table III "pericapillary round-cell infiltration". This change, illustrated in Figs. 5 and 6, comprises the aggregation of mononuclear cells round the smallest blood vessels to form cords or columns of cells with a small endothelium-lined lumen in the middle. Such cords of cells when cut in cross section may be mistaken by the uncritical for the groups of cells which persist for a short time at the site of a neurone which has undergone neuronophagia. However, careful examination usually reveals the endothelial lining and the central lumen (Fig. 6). The origin and nature of these cells has not been established with certainty; it is improbable that they are proliferated endothelial cells as their nuclei are quite different from the occasional endothelial cell nucleus visible at their centre. When this process has developed fully, the affected tissue is traversed by a branching and interlacing mass of these cellular cords, usually associated with a dense accumulation of round cells in the perivascular spaces of nearby larger vessels. This gives rise to a very striking appearance to which the name 'granulomatous lesion' may be applied. Such lesions are characteristic of attenuated viruses and are rarely if ever seen in cynomolgus monkeys inoculated with more virulent strains; good examples are shown in Figs. 1, 2, and 7, and in Fig. 6 of Pette et al. (1960).

No matter how early they are examined, it is seldom possible to distinguish any differences in the

FIG. 12. Cynomolgus monkey inoculated intraspinally with inactivated virus and killed 18 days later. Section to show absence of virus activity in damaged white matter. Contrast with Fig. 11. Galloycyanin. × 50.
age of lesions in different parts of the central nervous system of one animal if they are due to a virulent virus. However, this is not always the case with attenuated strains and several specimens have been observed in which such a difference was clearly apparent. For example, neuronophagia may be quite complete in the lumbar cord of an intraspinally inoculated monkey but only just beginning in the cervical cord or brain. Other features mentioned in Table II which characterize attenuated viruses are the production of sharply localized lesions (see Figs. 1, 2, 3, and 7) and their capacity to give rise to lesions on the posterior horn without lesions in the corresponding anterior horn at the same level (Fig. 2). Virulent strains may produce lesions in white matter, although such lesions are usually much less striking than those in neighbouring grey matter. Attenuated strains rarely produce more than minimal lesions in previously normal white matter, but are liable to do so in white matter damaged by mechanical trauma at the time of inoculation (Figs. 11 and 12).

It must be stressed that all the features listed in Table III will not be evident at every affected level of every positive monkey, and that the differences become less with time. However, provided that they are examined within about three weeks of the injection of virus (preferably sooner) it is possible to characterize the lesions in most of the affected monkeys as either of ‘virulent type’ or ‘attenuated type’. Some specimens, however, must be graded as ‘intermediate type’, either because they show within their lesions some of the characteristic features of both types or because they appear to have ‘attenuated type’ lesions at some levels, ‘virulent type’ lesions at others. The lesions in a proportion of animals have to remain ungraded because they are too trivial for the characteristic features to be discernible.

It is important to stress again that the neurovirulence of a particular strain of virus cannot be assessed by noting the character of the lesions it produces in a single monkey, but examination of a number of positive animals will reveal the usual behaviour of the particular virus. Because the features discussed in this section can only be recognized in animals with more than minimal lesions, it is necessary to use an adequate dose of virus and the intraspinal route of injection if the character of lesions is to be readily assessed.

**DISCUSSION AND CONCLUSIONS**

The foregoing account of the character, severity, extent, and distribution of lesions produced in monkeys by attenuated strains of poliovirus has necessarily emphasized those features which tend to distinguish them from those caused by the more virulent viruses used in the classical work on experimental poliomyelitis. In order to avoid giving too distorted a picture it is important to stress that the similarities are just as striking as the differences; the condition produced by the attenuated viruses is still recognizably poliomyelitis. Nevertheless the differences are real and are reproducible.

The essential difference between virulent and attenuated polioviruses is still unknown. However, the findings described do provide two possible clues. The restricted distribution of brain lesions seen in animals inoculated intraspinally with attenuated strains is strikingly similar to that described by Bodian and Howe (1940) in monkeys inoculated into the sciatic nerve with the virulent M.V. virus and killed at the time of the first detectable rise in temperature, which was judged to be about 24 hours before the time when paralysis would have developed. Reference has been made to the fact that it is sometimes possible to recognize age differences in the lesions in different parts of the nervous system of animals inoculated with attenuated virus strains, but practically never in animals infected by virulent strains. The first of these observations suggests that infection by attenuated strains is arrested at an earlier stage than infection by more virulent strains, and the second suggests that this may be due to slower spread of the virus rather than to arrest of the infection earlier in time. The absence of oedema, capillary haemorrhage, and more than scanty polymorphonuclear leucocytes together with more abundant mononuclear cells in the early stages of infection all point to the tissue injury being less acute than that due to virulent strains of virus. The remarkable absence of chromatolysis in surviving neurones in or close to the sites of neuronal destruction due to Sabin’s type 1 and type 2 strains was wholly unexpected, and its significance is not easy to assess.

The early arrest of the spread of infection makes it easier to study the pathways whereby the virus travels within the central nervous system. Virulent viruses spread so rapidly that by the time symptoms occur the infection is usually too widespread for it to be possible to determine the pathways of spread. The frequent involvement of the red nuclei, lateral, vestibular nuclei, roof nuclei of the cerebellum, dentate nuclei, lateral reticular nuclei, lateral cuneate nuclei, inferior olivary nuclei, and central reticular formation of the medulla to the virtual exclusion of the cranial motor nuclei might have been predicted from Bodian’s work (Bodian and Howe, 1940) but not from most descriptions of the disease. All the structures listed above are components of what may be called the cerebellar system.
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The only important component of this system which is not included in the list is the cerebellar cortex itself. Why it should not be involved more often is not clear. In the few animals which did show lesions in this situation, the changes resembled those described by Baker and Cornwell (1954). The distribution of 'solitary lesions' suggests that the pathways of most importance in the spread of virus from the lumbar cord to the brain are the rubro-spinal, vestibulospinal, and reticulo-spinal tracts. It can of course be argued that the distribution of lesions in intraspinaly inoculated monkeys is not relevant to natural infections in man. However, it is worth noticing that when brain lesions follow intramuscular inoculation of vaccine viruses their distribution is essentially the same as those seen in intraspinaly inoculated animals and that cerebellar signs do occur in human poliomyelitis (Wickman, 1907; Arthuis, Lyon, and Thieffry, 1960).

The association of certain pairs of lesions such as one red nucleus and the opposite dentate nucleus, but never the homolateral dentate nucleus, emphasizes the fact that the poliovirus travels by defined neural pathways and that it is not spread by the blood stream nor does it spread indiscriminately through the nervous tissue. A natural corollary of this is that all nerve cell groups are not equally accessible to virus inoculated at one point, and makes it clear that the distribution of lesions may be determined as much by the 'accessibility' as by the 'susceptibility' of the different cell groups. In man at least, cranial nerve palsies are more common in poliomyelitis than obvious cerebellar signs. This is explicable if we postulate that although less accessible than the cerebellar nuclei, the cranial motor nuclei are, in terms of neuronal destruction, more susceptible, once they are invaded by virus.

It is important to try to form some estimate of the extent to which the observed distribution of lesions in the experimental animals was determined by the choice of material for examination. Obviously a structure never examined will not appear in Tables I or II. It was appreciated that this would be a problem as soon as the study began. Practical considerations restricted the number of levels which could be examined. However, the levels for routine examination were selected on the basis of much more extensive examinations of earlier experimental monkeys inoculated with attenuated and with more virulent strains. Except for the cranial nuclei referred to in the footnote to Table I, all the structures listed were examined in virtually every animal. It is most unlikely that the incidence of poliomyelitis lesions in any structure not listed exceeds 1 to 2%, except possibly in the nucleus of the locus caeruleus and the principal sensory nucleus of the fifth nerve (type 3 virus only). However, because of the sharply limited nature of the lesions due to attenuated strains of poliovirus some of the rates given in the tables for the larger structures are probably too low. Although the sections will have shown one part of a particular nucleus, the lesion may have been in another and, therefore, not have been recorded.

There can be no doubt that the differences described are real ones and that they are reproducible. We have been fortunate enough to examine comparable specimens prepared by a number of other workers in Great Britain and North America. The characters described have been evident in all the material studied. Although the findings described in this paper relate mainly to animals infected with Sabin's strains of poliovirus, enough work has been done with other strains of low neurovirulence to indicate that the features mentioned are not peculiar to these strains, but, rather, are characteristic of all strains of comparable levels of neurovirulence.
T. S. L. Beswick, C. R. Coid, E. Hartley, Moira Henderson, and Maureen Winter

SUMMARY

The severity, character, and distribution of poliomyelitis lesions in monkeys inoculated with attenuated strains of poliovirus are described, with particular emphasis on the features which distinguish such lesions from those due to more virulent strains. Such differences are seen to be clear-cut and reproducible, although unsuitable as the basis for formal 'histological' markers.

There are definite differences between Sabin's three attenuated strains. However, it is not clear whether these represent differences in the level of attenuation or reveal essential biological differences between the three serotypes of poliovirus.

Our thanks are due to the technical staff for preparing the very large number of sections involved and for the day-to-day care of the monkeys. Professor Paul Glees of Göttingen has read the manuscript and we are grateful to him and to other workers in this field for helpful discussions while the paper was in preparation. Finally, we would record our indebtedness to those workers, too numerous to mention individually, who have allowed us to study their own material.

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