STUDIES ON THE ENTRY AND EGRESS OF POLIOMYELITIC INFECTION

I. Neutrotropic Infection of the Peripheral Ganglia in Apparently Healthy Monkeys Following Casual Exposure*

By HAROLD K. FABER, M.D., ROSALIE J. SILVERBERG, and LUTHER DONG

(From the Department of Pediatrics, Stanford University School of Medicine, San Francisco)

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The present communication deals with a purely morphological study of lesions occurring in certain peripheral ganglia of monkeys not intentionally exposed to infection but housed in our animal quarters for varying periods of time while experimental work with poliomyelitis virus was being actively prosecuted. No strict precautions were observed to isolate infected from other animals.

Until recently it was generally assumed that laboratory primates known to be susceptible to poliomyelitis by inoculation, do not acquire the disease from casual exposures in animal quarters in which infected animals are housed, and that this constitutes a basic difference between man and other primates in respect to susceptibility to poliomyelitic infection. Evidence that such a belief is not altogether correct was furnished by Howe and Bodian (1) who in 1944 reported clinically inapparent infection by accidental laboratory contagion in two chimpanzees, and again by Bodian (2) who in 1948 reported a paralytic case in an uninoculated *rhesus* monkey treated with desoxypyridoxine. In all these instances virus was recovered and identified from the affected animals, and the two chimpanzees were shown to be refractory to subsequent oral administration of infected feces.

For the last seven years we have been interested in the role of peripheral ganglia, particularly those of afferent cranial nerves and of the sympathetic system in relation to centripetal invasion of poliomyelitis virus from the body surfaces, especially the mucous membranes of the respiratory and alimentary passages (3–6). Granting, as now seems highly probable, that such peripheral nerve invasion constitutes the natural route of infection in most cases, it is anatomically necessary for the ingoing virus to pass through and presumably infect the regional ganglia. It is also conceivable that infection might stop at this point, without invasion of the CNS.

In 1943 we published observations (4) on a series of 4 uninoculated *cynomolgus* monkeys, used as controls for a study on the alimentary portal of entry, in

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one of which (C92), kept for several months in the laboratory and showing no symptoms of disease, many infiltrative and neuronophagic lesions were discovered in various peripheral ganglia, notably the gasserian, nodose, and cervical sympathetic, which we suspected might be due to poliomyelitis. McClure (7) had already noted the occurrence of lesions in peripheral ganglia both in inoculated and uninoculated *rhesus*, more frequently and of a more severe type in the former. Similar observations have been made, especially in chimpanzees, by Bodian and Howe (8). Since our first observations in 1942 (3), we have examined serial sections of the peripheral ganglia from a considerable number of uninoculated *rhesus* and *cynomolgus* monkeys, largely for purposes of control for other studies. Since lesions have been found in the ganglia of many of these, it has interested us to compare "new" animals, sacrificed within a few days of arrival with "old" animals kept for longer periods in our animal quarters next or near animals infected with poliomyelitis virus (*OH*, *Per*, and *Cam* strains). The present report gives the results of this comparison. The study includes no attempts to recover virus nor to determine the presence or absence of specific antibodies.

Material

For the present study, peripheral ganglia from 26 monkeys were examined by serial section. Of these, 9 (6 *rhesus*, 3 *cynomolgus*) were "new" and were sacrificed 1 to 3 days after arrival in the laboratory; 17 were "old" (11 *rhesus*, 6 *cynomolgus*) and were sacrificed 17 days to 10½ months (all but 2, more than 1 month) after arrival. Einarson's gallocyanin stain was used throughout. The gasserian, geniculate, petrosal, nodose, and superior cervical sympathetic ganglia were examined in all cases, and the celiac in 15. The CNS was not examined.

The pathological lesions consisted mainly of focal infiltrations and neuronophagia. Perivascular infiltrations were infrequent, occurring in 7 sympathetic ganglia from 5 monkeys and in 5 cranial nerve ganglia from 5; they were usually less heavy than those ordinarily encountered in the infected CNS. The predominant cells in the infiltrates were mononuclear. Polymorphonuclears were seen only in two instances. The infiltrations occurred both within and external to the perineuronal capsule, nearly always in the body of the ganglion. Infiltrates along the fiber tracts entering or leaving the ganglion were occasionally noted but have not been included in the tabulations. The small lymphoid nodules with germinal centers, noted by Bodian and Howe, were observed rarely by us; they can be easily distinguished from inflammatory lesions. Small calcified areas, sometimes surrounded by a ring of round cells, were occasionally seen and have not been included in the tabulations.

Chromatolysis in nerve cells of ganglia is difficult to determine because of the normally fine dispersion of the Nissl substance. For evidence of neuronal damage we have depended mainly upon the signs of neuronophagia, as follows; (1) a distinct capsule devoid of a nerve cell and filled with infiltrating cells, as followed in serial sections; (2) moderate sized or large infiltrations in which either no neurons were found, or only a few as compared with the distribution in the surrounding normal tissue and these often in necrotic remnants; (3) varying stages of active phagocytosis ranging from a few infiltrating cells within the capsule indenting the boundaries of the neuron to almost complete filling of the capsule, with acidophilic debris still visible. Neuronophagia is more easily determined in sensory than in sympathetic ganglia; the nerve cells in the former have more clearly defined capsules, the neurons are grouped more closely, and there is less interneuronal white matter and connective tissue.

For purposes of tabulation a somewhat arbitrary system of grading of lesions has been adopted, based on the sizes of the foci as compared with those of the perineuronal capsules. The smallest foci, graded as +, were 1 to 5 capsules or approximately 50 to 250 μ, in diameter. Those graded as ++ were 6 or more capsules ($\pm$ 300 μ) in diameter, while those graded +++ were the very large ones, roughly 15 or more capsules ($\pm$ 750 μ) in diameter. Neuronophagia has been designated as present or absent without any attempt to estimate the number of affected nerve cells.

In our earlier papers (4–6) we attempted to distinguish between "significant" and "insignificant" infiltrative lesions based on relative sizes, and Bodian and Howe (8) have made a similar distinction between "specific" and "non-specific" lesions. With increasing experience we have found that distinctions based on size have become increasingly unsatisfactory because a continuous gradation in size is to be observed between the most minute and the largest infiltrations, the cells of which they are composed being apparently of the same types. However, as will be presently seen, the *number* of lesions in a given ganglion appears to have a limited significance and the presence or absence of neuronophagia, a definite one. No significant difference between *rhesus* and *cynomolgus* ganglia was noted.

The geniculate and petrosal ganglia (mainly gustatory) showed no lesions of importance in any of the "new" or "old" monkeys, and the results are not tabulated.

In 3 of the "old" *cynomolgus*, kept in the laboratory 7, 10½, 10½ months respectively, thoracic and lumbar sympathetic ganglia and cervical, thoracic, and lumbar spinal ganglia were also examined, with mostly negative results, as follows:—

47 thoracic sympathetic ganglia, 43 negative, 4 (all in one animal) +; 27 lumbar sympathetic ganglia, 23 negative, 4 + (in 2 animals); 40 cervical spinal ganglia, all negative; 59 thoracic spinal ganglia, 58 negative, 1 +; 35 lumbar spinal ganglia, 34 negative, 1 +. No lesions larger than 1 + and no neuronophagia were found in any of the above. 7 of the 8 thoracic and lumbar sympathetic ganglia containing lesions were in C92 (*vide supra*).

RESULTS

The results of our observations on four different ganglia are condensed and tabulated in Table I.

The results in a group of 6 *cynomolgus* monkeys, also included in Table I, are separately tabulated in Table II, as being of special interest.

The animals belonged to a single lot imported from the Philippines, and were received on Oct. 7, 1946. They were collected between May and October, mainly on the island of Mindanao; assembled near Manila where they were kept 1 day; and flown to San Francisco on the Mars.[1] Three of the 6 were sacrificed on Oct. 9, 2 days after arrival.

The other 3 were intentionally placed in cages facing, and separated by a distance of about 2½ feet from, those of other animals of the same shipment which were fed virus (Cam strain) daily by capsule (for experiments already reported (9)) on Oct. 30–Nov. 1 Dec. 2–4, 1946, and Jan. 6–8, 1947. In pools of stools from these virus-fed animals, collected,

[1] By courtesy of the United States Navy.

Oct. 30–Nov. 3, Nov. 4–9, Dec. 8–12, 1946, and Jan. 6–10, 1947, virus was recovered. On the opposite side of the same room a group of 6 *cynomolgus* monkeys were fed virus mixed with their regular food on three occasions during the same period; Nov. 25, Dec. 17, 1946, and Jan. 12, 1947. During each of these feeding periods, one animal developed poliomyelitis (10). While the stools of these virus-fed animals were not examined for virus, it is known that the food in the cages was contaminated, and it is highly probable that they excreted virus for part of the time. The 3 uninoculated animals thus potentially exposed were sacrificed on Feb. 14, 1947, 4½ months after arrival. None had shown any evidence of disease.

TABLE I

Lesions in Peripheral Ganglia

26 uninoculated monkeys, *rhesus* and *cynomolgus:* 8 "new" and 17 "old"

Lesions	None		+		++, +++		Neuronophagia	
Ganglion	No. of animals	Per cent	No. of animals	Per cent	No of animals	Per cent	No. of animals	Per cent
Gasserian								
New (9)	4	44	5 [5.2]	56	0	0	0	0
Old (17)	2	12	15 [6.3]	88	4 [1.3]	24	7	41
Nodose								
New (9)	5	56	4 [3.0]	44	1	11	0	0
Old (17)	5	29	12 [3.0]	71	4 [1.5]	24	7	41
Superior cervical sympathetic								
New (9)	2	22	7 [3.0]	78	3 [1.0]	33	0	0
Old (17)	3	18	14 [6.2]	82	12 [2.1]	71	6	35
Celiac								
New (7)	3	43	4 [2.0]	57	1 [2.0]	14	0	0
Old (8)	3	38	5 [11.0]	62	3 [1.0]	38	0	0

New, in laboratory 3 days or less (6 *rhesus*, 3 *cynomolgus*).

Old, in laboratory 17 days or more (11 *rhesus*, 6 *cynomolgus*).

Numbers in parentheses, numbers of animals examined. Numbers in brackets, average number of lesions per animal.

This tabulation includes 3 new and 3 old *cynomolgus* separately tabulated in Table II, special group.

The results of histological examination of the ganglia in this group are shown in Table II.

While no examinations of the CNS were made in the present series, other, comparable observations indicate that "spontaneous" lesions in the brain stem of "old" animals are very infrequent. Thus, in 359 monkeys, (336 *rhesus*, 23 *cynomolgus*), all kept in the same laboratory quarters for a month or more, which had been inoculated intrathalamically with material suspected of containing poliomyelitis virus and which had failed to show overt signs of disease, the brain stem was examined by semiserial section (thalamus through medulla

at 1.25 mm. intervals) with completely negative results beyond the immediate region of the inoculum in 289, or 80.7 per cent. Of the 70 remaining with lesions of one kind or another in the brain stem below the inoculum, 17 showed evidence of probable or definite poliomyelitis "takes" from the inoculation, 8 showed subependymal perivascular infiltrations, 44 showed small areas of perivascular or parenchymal infiltration caudal to the inoculum insufficient to justify a firm diagnosis of poliomyelitis, and 1 had an abscess of the cerebellum. The majority of these last were probably due to the inoculations, but omitting

TABLE II

Lesions in Peripheral Ganglia

Cynomolgus monkeys, special group: 3 new and 3 old

Lesions	None		+		++, +++		Neuronophagia	
Ganglion	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent	No. of animals	Per cent
Gasserian								
New	2	67	1(4)	33	0	0	0	0
Old	0	0	3(11.3)	100	2(3)	67	3	100
Nodose								
New	1	33	2(3.5)	67	1(2)	33	0	0
Old	0	0	3(4.3)	100	1(2)	33	2	67
Superior cervical sympathetic								
New	0	0	3(2.3)	100	2(1.0)	67	0	0
Old	0	0	3(7.3)	100	3(1.7)	100	3	100
Celiac								
New	2	67	1(1)	33	1(2)	33	0	0
Old	1	33	2(2.5)	67	1(1)	33	0	0

New, 2 days. Old, 4½ months.

Figures in parentheses, average number of lesions per animal.

only those animals with a presumptive diagnosis of poliomyelitis from inoculated material, 85 per cent of the remaining 342 were free of brain stem lesions outside of the inoculated region in the thalamus. Comparing this percentage of negatives with the 65 per cent of "old" animals in the test series showing neuronophagia in the peripheral ganglia, we conclude that this lesion at least was mainly of centripetal rather than of centrifugal origin.[2] To the above we

[2] In previous papers (3–6) we have repeatedly called attention to the difficulty inherent in distinguishing centrifugal from centripetal origin of lesions in the ganglia. Bodian and Howe (8) have unfortunately misinterpreted our attitude in this matter, stating we "have concluded that centrifugal spread cannot occur from the CNS to the cranial nerve ganglia." We again wish to emphasize that we have been and are fully aware of the latter, which unquestionably

may add data from 12 *rhesus* monkeys (part of a study to be reported later) in which, 2 to 7 days after exposure of the central cut end of the infraorbital nerve to poliomyelitis virus, heavy lesions with neuronophagia were found in the gasserian ganglia and none in the CNS, the latter examined by semiserial section, with special attention to the trigeminal centers.

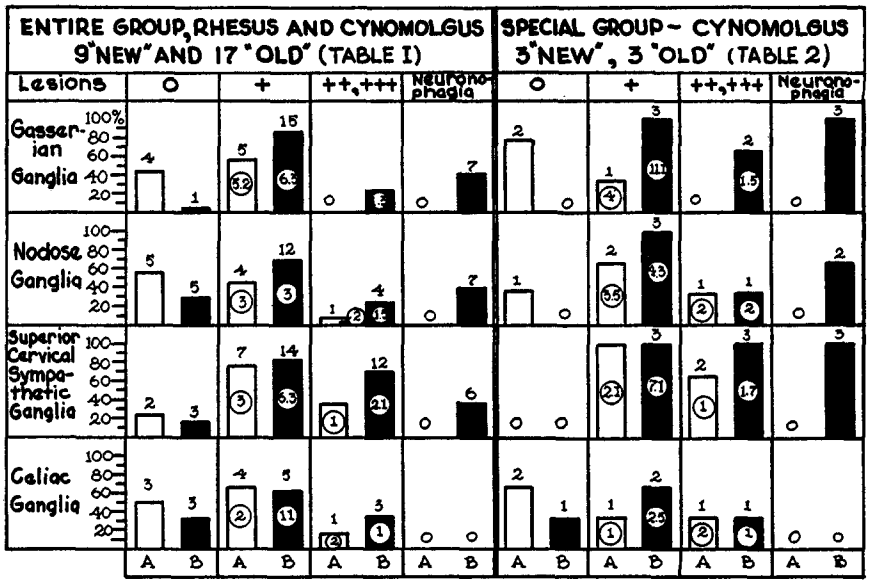

FIG. 1. Lesions in ganglia of "new" and "old" monkeys. A, "new;" B, "old." Heights of columns, percentages of total animals examined. Figures above columns, number of animals. Figures in circles in columns, average number of lesions per animal.

SUMMARY OF RESULTS

As shown in Tables I and II and in Fig. 1, minimal infiltrative lesions (+) were present in the ganglia of both "new" and "old" monkeys but the proportion of monkeys showing them and the average number of lesions were greater

occurs when the CNS is infected. However, we must insist that if poliomyelitis infection enters the body through peripheral afferent nerves supplying the surfaces it must of anatomical necessity pass through and presumably infect the corresponding ganglia before it reaches the CNS. Our own conclusions that the main entering pathways are from the oropharyngeal region through the regional nerve supply and perhaps occasionally from the intestine agree in principle with the hypothesis offered by Bodian and Howe (8) "that the V, VII, and IX cranial nerves, serving the oropharynx, and the X cranial nerve supplying the lower alimentary tract are the most likely routes of passage of virus to CNS, according to the present state of our knowledge." We should note a correction, however, in regard to the X cranial nerve, the afferent components of which supply not only the intestine but also the oropharynx, the esophagus and stomach, and the lower respiratory tract.

in the old than in the new. The proportion of monkeys having larger infiltrative lesions was much greater in the "old" than in the "new" monkeys. *Neuronophagia was found only in the "old" monkeys*, being present in 11, or 65 per cent of the latter in one or more of the ganglia (none in the celiac ganglia). Neuronophagia, therefore, constitutes a qualitative difference between the "new" and "old" monkeys in the present series. The gasserian and superior cervical sympathetic ganglia showed the heaviest involvement, the nodose (vagal afferent) less, and the celiac (intestinal sympathetic) still less. It is to be noted that the gasserian and superior cervical sympathetic supply the head area only, the nodose also supplies the lower respiratory and entire alimentary tracts, and the celiac the small intestine and proximal part of the large intestine.

DISCUSSION

There can be little or no question that during a prolonged sojourn without isolation precautions in quarters where poliomyelitis virus and no other was being extensively employed, a large proportion (roughly $\frac{2}{3}$) of uninoculated *rhesus* and *cynomolgus* monkeys acquired lesions in the peripheral ganglia supplying the mucous membranes, especially those of the upper respiratory and alimentary passages. While not positively identified as poliomyelitic, these lesions were morphologically indistinguishable from those of proved poliomyelitic origin and were clearly due to an infective neurotropic agent. There is also good reason to believe that they were of centripetal rather than of centrifugal origin.

We therefore suggest that the present observations can properly be taken as a small scale example of how from casual exposure, perhaps comparable with "natural" conditions in man, a large proportion of a given population can silently acquire neurotropic infection resembling poliomyelitis. The fact that, despite their frequency, such lesions so rarely lead to clinical manifestations suggests a strong tendency for infection of this type to remain "latent" (meaning, at a low level of activity) for a certain period, and not to progress centrally beyond the initial infective focus in the peripheral ganglia. We may have here an analogue to the natural history of human poliomyelitis with its high percentage of inapparent ("subclinical") cases (11).

SUMMARY

In *rhesus* and *cynomolgus* monkeys without signs or symptoms of poliomyelitis, a comparison of the incidence, numbers, size, and character of lesions in certain peripheral ganglia (gasserian, nodose, superior cervical sympathetic, and celiac) was made between 9 "new" animals sacrificed 1 to 3 days after arrival in a laboratory devoted exclusively to poliomyelitis research, and 17 "old" animals housed there without special isolation precautions for periods ranging from 17 days to $10\frac{1}{2}$ months. The comparison showed that the "old"

animals had more infiltrative lesions of various sizes than the "new" and that neuronophagia occurred in 65 per cent of the "old" animals as compared with none in the "new."

The heaviest and most frequent involvement occurred in the gasserian and superior cervical sympathetic ganglia, while that of the nodose (vagal afferent) ganglia was somewhat less, and that of the celiac ganglia was still less and without neurophagia. The ganglia of the VII and IX cranial nerves were also examined and showed no lesions of note.

Reasons are presented for believing that the lesions were of centripetal and not of centrifugal origin.

The lesions, while not positively identified as poliomyelitic, were of similar morphology, were presumably due to an infective neurotropic agent, and were acquired under conditions of potential exposure to poliomyelitis virus.

The possibility is suggested that the asymptomatic acquisition of neurotropic lesions in this group of casually exposed monkeys can be comparable to the acquisition of "subclinical" poliomyelitis in man.

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