LYMPHOID LESIONS IN POLIOMYELITIS*, ‡

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Interest in the pathology of poliomyelitis has been focused largely on the central nervous system, and its visceral manifestations have been less extensively investigated. Among the latter, the changes in lymphoid tissue in acute poliomyelitis have been relatively neglected. Observations made in a series of 50 autopsied cases of human poliomyelitis[1] suggested that lymphoid lesions may be of unappreciated importance in this disease, and stimulated us to experiment with mice which had been treated with agents damaging to lymphoid tissues.

MORPHOLOGIC OBSERVATIONS

The 50 cases studied were clinically and pathologically typical of sporadic or epidemic poliomyelitis from the neuropathologic viewpoint. Some of the material has been reviewed by authorities at the National Institutes of Health. No atypical or doubtful cases were included.

The microscopic slides were stained in a routine manner with hematoxylin and eosin. In a small number of cases phosphotungstic acid–hematoxylin stains were made.

A majority of the protocols stated that the mesenteric lymph nodes, solitary follicles, and Peyer's patches of the small and large intestines were prominent, enlarged, moist, succulent, and injected or congested. The spleen less commonly showed similar alterations, which were often obscured by congestion and softening of the red pulp. Microscopically 41 of the 50 cases (82 per cent) showed striking hyperplastic and inflammatory lesions of lymphoid tissue in the intestinal follicles, mesenteric nodes, and spleen. Sections of tonsils were not available. These findings were most frequent and well developed in children and when death followed a rather rapid course with development of bulbar paralysis.

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Under low power magnification marked enlargement of germinal centers was observed, due to edema which separated the cells, and to active degeneration and regeneration (Figs. 1 and 2). Cytologically, reticulum cells in the centers of such areas showed swelling, pyknosis, irregular staining, or karyorrhexis of their nuclei, with small or larger areas of necrobiosis and cellular debris. In the same regions pink coagulated protein-containing fluid was found with extravasated or hemolyzed erythrocytes. At times this fluid formed pink lakes in germinal centers of splenic or lymph node follicles. Phagocytosis of this material by surviving reticulum cells was often present.

Around the edges of the affected follicles active regeneration of reticulum cells was indicated by presence of numerous mitoses. The combined findings have been likened to the reaction in typhoid (1, 2); however, degenerative changes are more prominent in poliomyelitis, and the number and phagocytic activity of the endothelial leukocytes are less than in typhoid (Fig. 3). The washed-out appearance of germinal centers observed here and previously noted in experimental poliomyelitis (3) may in part be ascribed to the severe localized edema and fluid exudation which develop.

Close study rather commonly demonstrated numbers of binucleate reticulum cells and some multinucleated forms (Fig. 4). The latter closely resembled Warthin-Finkeldey giant cells as reported in measles and varicella (4).

At times later stages of the inflammatory process were observed. The germinal centers were shrunken, and the reticulum cells packed together in stellate or fusiform shapes simulating fibroblasts. Necrosis, giant cells, and mitoses were absent. Hyaline material was sometimes deposited between the germinal center cells, or the germinal center became small or indistinguishable. Lymphoblasts and lymphocytes did not appear to participate in any of these changes. The blood vessels showed enlargement of their endothelial nuclei, and proliferation and desquamation were found in lymphatic sinusoids. As a rule the thymus was unaffected.

In 5 of the 50 cases, intranuclear inclusions were identified in reticulum cells located at the margins of germinal centers. The inclusions were very scarce, eosinophilic, splinter-shaped, or spherical and surrounded by clear haloes. Nuclei which contained them showed margination of the nuclear chromatin (Figs. 5 and 6). Care was taken to exclude artefacts and confusion with nucleoli. The latter were usually basophilic and lacked clear haloes. As far as could be determined from illustrations these inclusions were like those found by Sabin (5, 6) in anterior horn cell nuclei. Similar inclusions were identified in the anterior horn cells of spinal cords from our human and mouse material. Aside from neurons and reticulum cells in lymphoid tissues, no other inclusions were found.

Two cases had acute myocarditis, and two others focal liver necrosis like that described elsewhere (1, 7–9).

Similar pathologic changes in lymphoid tissues in poliomyelitis have been described by Flexner, Peabody, and Draper (9) and Burrows (10). The latter found lymphoid hyperplasia so marked as to suggest that poliomyelitis might be primarily a disease of lymphoid tissues, only incidentally affecting the central nervous system. More recent articles have emphasized the non-specific nature of such findings, said to be common in lymphoid tissues in many acute infectious diseases of childhood (1, 11).

In order to investigate whether our findings dealt with non-specific reactions, lymphoid tissues were studied from a control series of various acute childhood inflammatory diseases including virus infections. Autopsy material from 7 pneumococcal, 2 meningococcal, 6 streptococcal, and 4 influenza bacillus meningitis cases, and examples of rabies, influenza, measles, measles encephalitis, virus hepatitis, and infectious encephalitis were examined, exceeding 50 in all. Comparable lymphoid lesions were found in one case each of measles encephalitis and meningococcal meningitis; aside from Negri bodies, no other inclusions were observed.

Taken together, the lymphoid lesions found in poliomyelitis resembled morphologically those described in other virus diseases. However, because in infectious disease morphologic findings often are not completely satisfying alone, an experimental study was initiated.

EXPERIMENTAL OBSERVATIONS

The effects of treating Swiss albino mice with agents damaging to lymphoid tissues prior to exposure to poliomyelitis virus were investigated.

All mice used were of the same Swiss albino stock, and of uniform age and weight. Best results were obtained using immature animals about 8 gm. in weight, and in a few instances mice up to 15 gm. Groups of 50 mice were handled in each experiment, except when sufficient chemical material was not available.

The strain of mouse-adapted human poliomyelitis virus MEF (Middle Eastern Forces (12)) proved very convenient, since after intracerebral inoculation it produced fatal paralysis in 211 of 212 mice after 2 to 17 days. Peak incidence of paralysis was on the 6th day, a much shorter interval than required in the case of Lansing poliomyelitis strains. MEF virus was obtained by removal with sterile precautions of brains and spinal cords from moribund paralyzed animals sacrificed by etherization, and was preserved in sealed sterile bottles frozen in liquid air. Virus emulsions injected contained 10 per cent brain tissue macerated with sterile physiologic saline. Standard doses of 0.03 cc. were injected intracerebrally, 0.1 cc. of virus emulsion intraperitoneally, and 0.05 cc. introduced orally. In each case, cultures in nutrient broth were prepared with virus emulsions, incubated, and examined to exclude the possibility of bacterial contamination.

Intraperitoneal injection of MEF virus alone is ordinarily ineffective. Localized brain irritation was produced by intracerebral injection of 0.03 cc. of 2 per cent corn starch emulsion (13). Four of 51 control mice receiving intraperitoneal virus and intracerebral corn starch developed paralytic poliomyelitis, an incidence of 8 per cent.

After administration of virus by this route, mice were examined daily for signs of paralysis. When moribund the animals were etherized, and the brains and spinal cords of each were

removed. In each experiment some material so obtained was emulsified and 10 per cent saline suspensions injected intracerebrally into groups of at least 5 mice, which were observed for development of paralysis. Other brains from infected mice were examined microscopically for the irregularly placed focal lesions in brain and spinal cord, with necrosis and phagocytosis of nerve cells, polymorphonuclear exudation, and intranuclear neuron inclusions considered typical of poliomyelitis. In each instance poliomyelitis infections were identified both by morphologic study and by injection of infected material into new mice followed by microscopic identification of poliomyelitis lesions in the recipient mice.

Since other virus work was proceeding in the same laboratory, all equipment used in poliomyelitis injections was autoclaved after use, and handled separately. Mice were caged separately from other experimental animals, and constant watch for signs of contaminant virus infections was kept. No evidence of spontaneous or accidental passage of other viruses was observed.

X-ray irradiation of the whole body, aminopterin (4-aminopteroylglutamic acid), and adrenocorticotropic hormone were the agents employed to cause lymphoid damage.

X-ray doses of 450 r were at first used, but since this lay in the range of LD_{50} to LD_{80} per 30 days, radiation sickness and deaths complicated the observations (14). The dose of 350 r caused only 1 death among 30 control mice, and was found preferable. Irradiation was carried out under the following conditions: 200 kv., 25 ma., 85 cm. focal skin distance, 1 mm. copper filter, given at 17 to 21 r per minute. Mice were irradiated in groups of 50 in a shallow covered cardboard box suspended so as to minimize back-scatter radiation.[2]

Aminopterin was given to one group of 50 mice intraperitoneally, in a dose of 0.005 mg. in 0.2 cc. of physiologic saline. Other groups received 0.02 mg. divided over a period of 13 days, in an attempt to produce chronic toxic effects. Two of 50 control mice given aminopterin alone died.

Sufficient adrenocorticotropic hormone (ACTH) was available for only two small scale experiments.[3] It was administered in 1 mg. amount in 0.2 cc. physiologic saline subcutaneously to each mouse. Control mice receiving ACTH alone all survived.

In the case of mice treated with x-ray and aminopterin, virus was administered 24 hours after the procedure administered to damage lymphoid tissue, and 6 hours after ACTH injection.

All three of the agents under test caused an increased incidence of poliomyelitis transmission by intraperitoneal injection. X-radiation, and irritation caused by intracerebral injection of corn starch produced a total of 30 proved poliomyelitis infections among 118 mice (25 per cent). There were 15 other deaths not preceded by paralysis, most likely due to poliomyelitis but not proved to be so on account of postmortem degeneration and bacterial contamination of brain material. The gross mortality was thus 38 per cent, excluding 21 deaths due to corn starch injection or brain damage among a total of 139 mice. Paralyses occurred from the 3rd to the 29th day after virus inoculation, most frequently in from 7 to 17 days.

[2] Appreciation is expressed to Dr. James A. Read and Dr. Donald R. Bryant of the X-ray Therapy Department, Henry Ford Hospital, who administered the radiation.

[3] We acknowledge the help of Dr. Ralph R. Margulis of the Gynecology Department, Henry Ford Hospital, who provided the ACTH.

Aminopterin, intracerebral corn starch, and intraperitoneal virus led to 5 paralyses and 5 deaths among 39 mice, a 26 per cent gross mortality ascribable to poliomyelitis. With ACTH, intracerebral corn starch, and intraperitoneal MEF virus, 5 of 19 mice or approximately 25 per cent developed poliomyelitis.

In contrast no success attended attempts to produce poliomyelitis by intraoral virus administration combined with the influence of the three agents mentioned. Only deaths ascribable to inhalation pneumonia were observed among a total of 69 mice. Treatment with x-radiation up to 450 r or with 0.005 mg. of aminopterin did not allow evident virus passage. Other workers have successfully employed bacterial toxins for this purpose (15), and the importance of post-tonsillectomy poliomyelitis in human beings is well known.

All mice surviving these experiments were tested for possible immunity to poliomyelitis, as follows: Into some animals MEF virus was injected intracerebrally, in 0.6 and 10 per cent suspensions. Others were splenectomized, the spleens emulsified with 0.6 per cent virus suspensions, and allowed to stand 1 to 2 hours at room temperature. The mixture was then centrifuged, and the supernatant injected intracerebrally in amounts of 0.03 cc. Neither method yielded any evidence of resistance to poliomyelitis virus, although a few mice in the group injected with virus exposed to spleen emulsion developed nonfatal paralyses simulating human poliomyelitis. This was not observed in any other experiments with Lansing or MEF strains.

Attempts to demonstrate virus in the spleens of mice previously injected intracerebrally with MEF virus also failed. Fifteen mice were splenectomized 1 to 4 days after intracerebral injections, and emulsified spleen from each day's group was injected intracerebrally into each of 5 mice. No poliomyelitis developed. All 15 splenectomized mice succumbed to paralytic poliomyelitis.

DISCUSSION

One major difficulty in both morphologic and experimental studies of poliomyelitis is the presence of unexplained variations in the action of the virus, at times appearing almost fortuitous. Yet several different approaches (10, 16–18) have suggested that in prodromal and fulminating human poliomyelitis there may be systemic dissemination of virus which becomes less considerable by the time paralysis is well established. The present reemphasis of lymphoid abnormalities in autopsied poliomyelitis cases is based on observing that aside from the central nervous system the lymphoid tissues exhibited the most frequent and marked changes. The coexistence of marked reticulo-endothelial degeneration and regeneration with giant cell formation, transudation of fluid into germinal centers, and rare inclusion bodies is consistent with a virus etiology of these lesions. Since lymphoid tissues are apparently a major source of antibodies, the difficulties of ourselves and others in extracting active virus from this source are not entirely unexpected.

X-radiation, aminopterin, and ACTH in mice could be used to increase the

susceptibility of the animals to mouse-adapted human poliomyelitis virus injected intraperitoneally. This suggests that lymphoid tissues may participate in resistance to poliomyelitis. Since all the toxic agents employed have generalized body effects, lowered resistance may not have been dependent upon lymphoid damage alone. However, the similar results with three agents having different types of action support the idea that all preponderantly affected the lymphoid tissues. Whether this damage interfered with the development of immunity in the mice is uncertain.

Failures of infection by oral administration of virus together with adjuvant influence of intracerebrally injected corn starch imply the existence of more than one defense barrier to poliomyelitis.

Using various accretions of knowledge of human or animal body responses to poliomyelitis virus, there is evidence for the existence of the following defense barriers:—

(*a*) Gastro-intestinal. This appears to be the first and most effective defense in mice and monkeys, probably also in most human beings (2, 6, 19). It is weakened by bacterial toxins (2, 15) or infection, by trauma such as stretching (2)) or excoriation (21, 22), and is bypassed by intramural virus injection (3).

(*b*) Lymphoid. As indicated, these tissues react vigorously when virus reaches the peritoneal cavity, mesenteric nodes, and spleen. Though rather severely damaged, lymphoid tissues appear to inactivate local virus effectively, and may contribute circulating substances of antibody type to neutralize virus elsewhere.

(*c*) Nervous tissue. The potentiating effect of experimental use of intracerebrally injected corn starch and investigations of human poliomyelitis after vaccinations (23, 24) point to the importance of a *locus minoris resistantiae* of traumatic or diverse origin in allowing poliomyelitis virus to localize in the central nervous system. Penetration of this barrier, unlike that of the first two, produces clinical poliomyelitis.

(*d*) Nerve cell. The irregular localization of poliomyelitis lesions implies that some neurons successfully resist the virus. Development of virus inclusions has also been suspected of indicating cellular immunity and interference with virus multiplication (25). Compared with the other defenses, the last appears relatively weak.

The opportunity to attempt primary isolation of human poliomyelitis virus by the use of irradiated mice did not arise, but appears worthy of trial.

SUMMARY

Examination of 50 autopsied cases of human poliomyelitis showed prominent hyperplastic and inflammatory changes in the lymphoid tissues of 41, the most frequent and severe lesions observed save those in the central nervous system. Histologically the germinal centers showed prominent degenerative and re-

generative alterations, fluid transudation, giant cell formation, and rare inclusion bodies, all consistent with virus effects.

Treatment of mice with x-rays, aminopterin, and adrenocorticotropic hormone increased poliomyelitis infections following intraperitoneal injection of MEF strain virus, potentiated by intracerebrally injected corn starch. This was ascribed to the damage to lymphoid tissues produced by these agents. On the basis of combined morphologic and virus studies, the presence of gastrointestinal, lymphoid, nervous tissue, and nerve cell defense barriers to poliomyelitis virus is suggested. Use of irradiated mice might prove useful in primary isolation of virus from human poliomyelitis.

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EXPLANATION OF PLATE 46

FIG. 1. Enlarged lymphoid follicle of colon with prominent germinal center. Acute bulbar poliomyelitis, male, age 14 years. A-5356, hematoxylin and eosin stain. × 120.

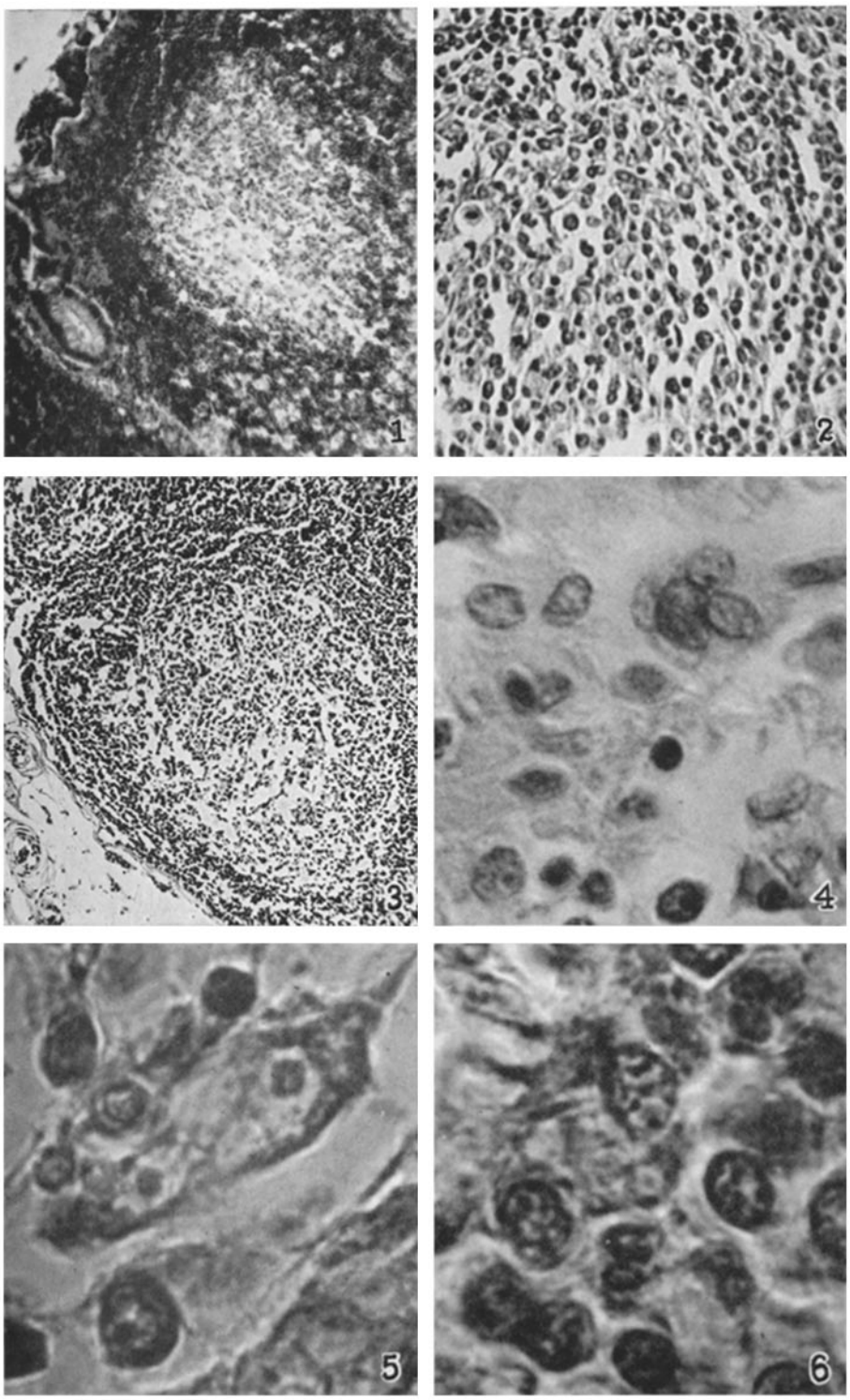
FIG. 2. Edema and necrobiosis in the germinal center of a splenic follicle. Poliomyelitis, male, age 5 years. H. K. H., A-455-11, hematoxylin and eosin. × 350.

FIG. 3. Marked destruction of germinal centers in mesenteric lymph node. Poliomyelitis, male child. A-2537, hematoxylin and eosin. × 75.

FIG. 4. Detail of germinal center shown in Fig. 1, with multinucleated giant cell. A-5356, hematoxylin and eosin. × 800.

FIG. 5. Enlargement of part of the germinal center shown in Fig. 2. Intranuclear inclusions present. H. K. H., A-455-11, hematoxylin and eosin. × 1200.

FIG. 6. Intranuclear inclusion in splenic reticulum cell. Poliomyelitis, male, age 7 years. H. K. H., A-2131-9, hematoxylin and eosin. × 1200.



(Sommers *et al.*: Lymphoid lesions in poliomyelitis)