The weight required to fracture the two femurs of the same mouse varied in most animals. These variations probably represent technical inaccuracies and biological fluctuations. The differences in breaking strength of the 2 femurs of individual estrogen-treated mice averaged 207 g and of the individual controls 147 g, ranging from 0 to 455 and 15 to 275 g.

The length and smallest diameter of the bones were determined by micrometer measurements prior to fracture. The bones of the test mice were approximately 1 mm shorter than those of the controls. The diameters were similar. All the control mice were in excellent general health. The treated mice showed the usual toxic effects of chronic estrogenic stimulation and most of them had tumors of the mammary glands, pituitary gland or both.

Summary. The average breaking-strength of femurs of estrogen-treated mice was 2499 g or 844 g greater than that of untreated controls.

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Influence of Sterile Inflammation on Susceptibility to Experimental Poliomyelitis.*

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Since neither the serum antibodies nor specific neutralizing substances in the nasopharynx[1] seem to present an adequate basis for the relatively high immunity to poliomyelitis, a further study of the mechanism of this resistance might be made by approaching the problem from the point of view of possible elements, other than the virus, as determining factors in establishing the infection. Is some symbiotic agent, a state of allergy, or an inflammatory condition of the mucosa with resulting increased permeability, a contributory factor in producing clinical cases of the disease?

Flexner and Amoss[2] reported that poliomyelitis could be produced successfully in monkeys with subinfective doses of the virus given

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1 Park, W. H., *J. A. M. A.*, 1932, **99**, 1050; Howitt, B. F., *J. Inf. Dis.*, 1937, **60**, 113.

2 Flexner, Simon, and Amoss, H. L., *J. Exp. Med.*, 1914, **20**, 249; Flexner, Simon, and Amoss, H. L., *J. Exp. Med.*, 1917, **25**, 1525.

intravenously, intranasally, or in the subcutaneous tissues, provided sterile inflammation of the meninges had been incited by the intraspinal injection of normal horse serum or even an isotonic solution such as Ringer's or Locke's solution. They found that a simple lumbar puncture with replacement of the cerebrospinal fluid of one monkey with that from another, or the slightest hemorrhage resulting from the lumbar puncture promoted infection. The controls in their experiments were relatively few, especially when one remembers the inconstancy of the methods of infection employed.

Zwick, Seifried and Witte[3] reported consistent success in the cutaneous infection of rabbits with virus of Borna disease only when injury to the central nervous system had been effected by the injection of non-specific substances such as normal rabbit or normal horse serum.

Sawyer[4] injected sterile starch solution intracerebrally into mice, thereby causing a locus of lowered resistance so that an intraperitoneal injection of a viscerotropic strain of yellow fever virus produced an encephalitis. It has been suggested that the aseptic inflammation produced by these procedures increases the permeability of the blood-brain barrier, thus permitting virus to pass into the nervous tissues.

If an increased susceptibility to intravenous inoculation can be induced by a sterile inflammation of the central nervous system, and if this change is due to an increased permeability of the blood brain barrier, the olfactory tract would not necessarily be involved in such a process. However, Lennette and Hudson[5] have reported that section of the olfactory tracts protected against intravenous inoculation, but that a subsequent intracerebral injection of starch rendered the same animals susceptible to subinfective intravenous doses of virus. These investigators demonstrated poliomyelitis virus in the nasal secretions of one monkey with olfactory tract sectioned about 24 hours after this animal had received virus intravenously, and, on this basis, suggested that in normal animals, even after intravenous inoculation, virus was excreted onto the olfactory mucosa, finally reaching the brain by way of the olfactory nerves. Furthermore, Armstrong[6] presents evidence that treatment of the nasal mucosa with picric acid protects monkeys against intravenous inoculation.

[3] Zwick, Seifried, and Witte, *Arch. wissensch. u. prakt. Tierheilk.*, 1929, **59**, 511.

[4] Sawyer, W. A., *J. Exp. Med.*, 1931, **54**, 533.

[5] Lennette, E. H., and Hudson, N. P., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 470; Lennette, E. H., and Hudson, N. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1444.

[6] Armstrong, Charles, *Pub. Health Rep.*, 1936, **51**, 241.

These findings and hypotheses led us to study anew the relation of sterile inflammation of the central nervous system to the portals of entry in the experimental disease; some of these results were reported briefly at the 1938 meetings[7] of the Society of American Bacteriologists.

Experimental Work. Sterile Inflammation and Infection by the Intravenous Route. In Series I, the olfactory tracts of 4 *Macacus rhesus* monkeys were sectioned surgically by Dr. K. E. Lemmer of the Department of Surgery. After allowing an interval of 3 or 4 weeks for recovery, sterile inflammation was produced in 2 of these animals by injecting 2 cc of sterile horse serum intrathecally and 1 cc of a 2% starch solution into the right frontal lobe of the brain; in Series II and III, 1% starch was injected into both frontal lobes. Each animal then received 10 cc of a 5% brain-cord suspension intravenously on each of 3 successive days, beginning on the day following the injections made for the production of sterile inflammation. Those animals with a sterile inflammation developed the experimental disease, while 2 animals with the olfactory tracts sectioned and 2 normal controls remained well.

A second similar experiment, Series II, with intravenous injection of 55 cc virus suspension, confirmed these results, save that one monkey with sectioned olfactory tracts and with sterile inflammation did not develop poliomyelitis. This animal had been used in Series I, and might, we thought at the time, have had some degree of immunity due to the earlier treatment. We have never found, however, any immunity to actual infection as the result of previous unsuccessful use of monkeys in experimental poliomyelitis, and in this case, subsequent intracerebral injection produced typical paralysis.

The results of these experiments seemed quite clear; an intravenous dose of virus, subinfective for 4 normal controls and 4 animals with olfactory tracts sectioned, incited the disease in 3 out of 4 monkeys with artificial sterile inflammation of the central nervous system even though the olfactory tracts were sectioned.

In the next experiment, Series III, infection by the nasal route was blocked by 4 treatments of the nasal mucosa with 1% zinc sulphate at 12-hour intervals. Twenty-four hours after the last instillation of the zinc salt, sterile inflammation was produced in 2 of these animals and in 2 normal animals by the method described. Upon intravenous inoculation of approximately 10 cc virus suspension per kilo, 3 of 4 animals treated with zinc sulphate developed polio-

[7] Clark, Lemmer, and Rasmussen, *J. Bact.*, 1938, **36**, 290.

myelitis; the fourth died an accidental death. One sterile inflammation control, without zinc sulphate treatment, showed no symptoms; the other died an accidental death. Of the normal controls, one developed the disease, the other died of tuberculosis 21 days after inoculation.

These results do not agree with the hypothesis that after intravenous inoculation, infection is by way of the olfactory nerve. It is evident that infection by the intravenous route may take place in animals in which the olfactory portal of entry has been blocked either by section of the olfactory tracts or by nasal instillation of zinc sulphate, as has recently been shown also by German and Trask[8] and by Toomey.[9] It is apparent that a sterile inflammation of the nervous tissues decreases the resistance to invasion from the blood stream, but the results reported here emphasize that many of the factors contributing to infection or resistance to infection in the monkey are still unknown. Schaeffer and Muckenfuss[10] have shown that the distribution of virus after intracerebral inoculation is a variable factor which may explain erratic results, particularly when small doses of virus are used. The distribution of starch after intracerebral injection must show a similar variation, with a consequent variation in the degree of injury and inflammation produced. Nor, as King[11] recently emphasized, has it been definitely shown that the inflammatory procedures act solely by increasing the permeability of the blood-brain barrier. The injury to the nerve cells, particularly in Sawyer's method, may be equally important.

The Local Effect of Zinc Sulphate. Schultz[12] has shown that after zinc sulphate treatment the olfactory mucosa is desquamated. Within 4 or 5 days the epithelium regenerates; but it is assumed that the protection, which lasts 8 to 12 weeks, persists until the olfactory nerve endings are regenerated. The protective action is thought to be localized in the olfactory mucosa. If the increased resistance were due to some more deepseated change, it might be expected that inflammation of the central nervous system would alter the effect of the zinc sulphate.

Four monkeys, Series IV, were treated 4 times at 12-hour intervals with about 1.5 cc of a 1% aqueous solution of zinc sulphate introduced a drop at a time. A sterile inflammation was then produced in 2 of these monkeys in the manner described; and all 4,

8 German, W. J., and Trask, J. D., *J. Exp. Med.*, 1938, **68**, 125.

9 Toomey, J. A., *Am. J. Dis. Child.*, 1939, **57**, 338.

10 Schaeffer, J., and Muckenfuss, R. S., *Am. J. Path.*, 1938, **14**, 227.

11 King, L. S., *J. A. M. A.*, 1939, **113**, 1940.

12 Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 603.

together with normal controls, were inoculated with poliomyelitis virus by dropping about 1.5 cc of a 5% milky virus suspension into each nostril 3 times at 12-hour intervals. All the zinc sulphate-treated animals with or without inflammation, were resistant. Series V is a duplicate of this experiment, giving the same results.

These results tend to confirm the hypothesis of Schultz that the action of zinc sulphate is localized in the olfactory mucosa.

Attempted Infection by the Gastrointestinal Route. The possibility that the increase in susceptibility due to sterile inflammation of the central nervous system might alter the resistance of the monkey to infection by the gastrointestinal route suggested the next experiment, Series VI. To 2 animals with sterile inflammation and to 2 controls, 25 cc of virus were given by stomach tube on each of 3 successive days. All of these animals remained well. As has been pointed out, the increase in susceptibility due to sterile inflammation of the central nervous system may be slight; and hence, this experiment cannot be regarded as definite evidence against the gastrointestinal portal of entry. It shows merely that the inflammation was insufficient to change the monkey's resistance to infection in this experiment.

Summary and Conclusions. 1. Following surgical section of the olfactory tracts or treatment of the nasal mucosa with zinc sulphate, the monkey can still be infected by intravenous inoculation, whether or not a sterile inflammation of the central nervous system is produced. 2. An increase in susceptibility to intravenous inoculation in monkeys with a sterile inflammation of the central nervous system is indicated by the experiments; variation in susceptibility in individual animals is always, however, an important factor. 3. The failure of sterile inflammation of the central nervous system to alter the protective action of nasal treatment with zinc sulphate suggests that the action of zinc sulphate is localized in the olfactory mucosa. 4. The resistance of the monkey to poliomyelitis infection by the gastrointestinal route was not sufficiently altered by a sterile inflammation of the central nervous system to produce the disease.