pattern is obtained in this study. Routh and Paul(8) in a similar study on anterior poliomyelitis obtained electrophoretic patterns which bear some similarity to our cases of multiple sclerosis. The similar distribution of protein fractions in these 2 dissimilar types of neurological lesions is to be noted, and the systemic change manifested by an increased a_2-globulin fraction, exhibits a uniformity which may be significant, and such studies should be extended to other types of neurological disturbances. The fact that a biochemical aberration can be demonstrated in the blood in neurological disorders is of some importance in further investigations on the behavior of nervous tissue. While this aberration is probably secondary in nature, the causative elements of such a finding should not be ignored.

Summary. 1. Electrophoretic fractionation of serum proteins in 27 cases of multiple sclerosis showed an increase in the a_2-globulin moiety with a concomitant reduction in the albumin component. This resulted in a lowered A/G ratio in the pathological cases. These differences were statistically significant. 2. In 15 out of the 27 cases an electrophoretic heterogeneity in the a_2-globulin fraction was observed which was characterized by a "double" peak.

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Association of Group A Streptococci with an Outbreak of Cervical Lymphadenitis in Mice. (21159)

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In October, 1953 a suppurative involvement of the neck was observed in Princeton mice by Dr. Alice E. Moore at the Sloan-Kettering Institute. These animals had been injected subcutaneously some weeks earlier with bacteriologically sterile suspensions in connection with her work on the suppression of tumor growth by viruses. Since the cervical reaction was in no way related to the activity of the injected agents a number of the affected mice were brought to the laboratories of animal pathology at the Rockefeller Institute for examination. The Princeton strain, which was originally established in these laboratories, has been under observation for many years. The mice used by Dr. Moore were obtained, however, from a commercial colony.

The signs of disease were unlike any previously observed in Princeton mice. The under surface of the neck showed eroded areas, with loss of hair and matting of the hair by a sticky exudate. In some animals the cervical lymph nodes were prominent and palpable. The mice were sickly in appearance and several that were held under observation died within a few days.

Fifteen of the diseased mice were killed and autopsied. The diagnosis in all of them was purulent cervical lymphadenitis. It resembled the condition originally described in guinea pigs by Boxmeyer(1) and sometimes known as "lumps" but was less progressive. In advanced cases one or more of the abscessed cervical nodes ruptured, releasing a semi-fluid purulent exudate. Its volume was evidently increased by subsequent involvement of the

adjoining tissues. Perforation of the skin ultimately occurred with drainage of the fluid through a clean-edged hole.

A number of the mice also showed unmistakable evidence of infectious catarrh, and pleuropneumonia-like organisms (PPLO) were isolated from the middle ears. In addition, these organisms were recovered from the external cervical exudate of 2 animals, presumably as secondary invaders acquired by contact.

Stained films of exudate from the involved lymph nodes invariably showed gram-positive cocci and colonies of a hemolytic streptococcus were regularly obtained on horse blood-agar plates. Dr. Rebecca Lancefield kindly identified the organism and found it to be a group A streptococcus. It did not belong, however, to any of the known types on the basis of the M precipitin reaction.

The experimental production of streptococcal cervical lymphadenitis in mice was reported by Sonkin(2). He described a technic for the deposition of inocula on the nasal mucosa in such a manner that they were confined there and did not flow to other portions of the respiratory tract. The introduction of group C streptococci by this method was attended by a high rate of cervical lymph node inflammation, 98% in 161 mice. On direct pulmonary injection, by way of the trachea, the rate was much reduced, 2.3% in 42 mice. A fatal pneumonia occurred in all of the animals of both series. Glaser, Berry, and Loeb (3) employed a modification of this method with group A streptococci. Their mice were first injected intraperitoneally with chloral hydrate and then anesthetized with ether. They also obtained a high rate of cervical lymphadenitis. The infection was generally fatal within 120 hours. Webster's selected strain of Swiss mice was used in both of these investigations.

Methods. The pathogenicity of the group A streptococcus obtained from mice was determined by the nasal introduction of recently isolated pure cultures. Princeton and Swiss mice (the original, unselected, Rockefeller Institute strain) were used in these tests. Most of the injections were made in weanlings, 4 to 5 weeks old (10-15 g in weight), but adults, 8 to 12 months old (25-50 g in weight) were also employed.

The streptococcus was generally isolated from lymph node exudate and transferred in culture not more than 5 times. It was grown in 1.0 ml of 10% horse serum-heart infusion bouillon (pH 7.4) at the base of slanted nutrient agar. Six- to 7-hour growths at 37°C were generally used but occasionally the culture was incubated up to 18 hours. The fluid was removed and diluted with an equal volume of saline. The slant was reincubated and then preserved as a stock culture.

The mice were anesthetized with ether in an open battery jar. Prior injection of chloral hydrate was also tried but was not superior to ether alone. The inoculum was introduced by one of 2 methods referred to as nasal inhalation and nasal deposition. The first method entailed placing about .02 ml of diluted culture dropwise on the nares of weanlings and up to .05 ml with adults. A Luer syringe (¼ ml) with a 24-gauge needle was used. With the second method a special 30-gauge needle delivering a minute drop, .01 ml or less, was employed. The needle was inserted a short distance into one nasal passage and a single drop expelled. This method was less precise than that used by Sonkin(2) but serviceable. The 6- to 7-hour cultures of the streptococcus contained between 6×10^8 and 8×10^8 organisms per ml.

The mice were generally injected in groups of 5, either sex being used. Most of the animals that died were autopsied. All survivors were killed after the 7th to the 14th day and examined. When indicated, Gram-stained films and blood agar cultures were made from the lungs and lymph nodes. The middle ears and nasal passages of all survivors were exposed and aspirated with a capillary pipette. If exudate was present, blood agar cultures and films were made. In a few instances 20% horse serum-agar plates containing 2500 units of penicillin were also inoculated for PPLO determination.

Results. The experimental findings, summarized in Table I, indicated a difference in the susceptibility of Princeton and Swiss weanlings to the streptococcus. These 2 mouse strains are known to vary in their re-

TABLE I. Response of Princeton and Swiss Mice to Nasal Introduction of Group A Streptococcus.

Strain and age of mouse	Method of introduction	No. of mice	No. of deaths*	No. with adenitis
Swiss weanlings	Inhal.†	25	9	2
Princeton "	"	"	23	0
Swiss "	Depos.	"	1	2
Princeton "	"	"	2	18
Swiss adults	Inhal.	"	20	2
Princeton "	"	"	21	2

* All deaths occurred between the 2nd and the 7th day.
† Inhal. = Inhalation. Depos. = Deposition.

sponse to other microbial agents, as the virus of murine hepatitis(4). Princeton weanlings infected by nasal deposition were the only mice in which cervical lymphadenitis was reproduced with any degree of regularity. The morbidity rate was 72%, in comparison with 8.0% in Swiss mice infected in the same way. The reaction was commonly unilateral, corresponding to the site of deposition. It varied from focal inflammation to nearly complete replacement of the node by a purulent exudate. Release of the exudate by rupture left a thin shell of lymphoid tissue and was ultimately followed by perforation of the skin. Hemolytic streptococci were commonly recovered in pure culture from the involved nodes. The morbidity rate was considerably less than that reported by Sonkin(2) for group C streptococci but supported his conclusions on the efficacy of nasal deposition in localizing the organisms.

Nasal inhalation was more commonly followed by carriage of the streptococci to the lung. The resulting pneumonia was diffuse in distribution, often involving parts of all 5 lobes. Stained films of lung tissue showed innumerable gram-positive cocci and hemolytic colonies were obtained on blood agar. The mortality rate was much higher in Princeton than in Swiss weanlings, 92 as compared with 36%. Immature and adult Princeton mice were about equally susceptible to pneumonia. In Swiss mice, however, the adults showed a higher death rate than did the weanlings. The adult mice available were too few in number to determine the effect of age on the outcome of nasal deposition.

In connection with long continued studies, in these laboratories, on murine otitis media caused by PPLO it was of particular interest that group A streptococci also tended to involve the middle ears. In the present series otitis media was rarely accompanied by rhinitis, as it commonly is in mice infected nasally with PPLO. Only 3 mice, all adults, showed unmistakable inflammation of the nasal passages. Middle ear examination was restricted to mice that survived at least 7 days after nasal inhalation or deposition. The incidence of otitis media in 45 Princeton weanlings was 62% (28 cases) and in 55 Swiss weanlings 72% (40 cases). Streptococci were demonstrable in exudate films or cultures from all but 2 of these mice. Two Princeton mice of this series, which includes animals not listed in Table I, and 6 Swiss were "twisters." Under usual circumstances this condition is rarely seen in mice. The head is inclined to one side; the affected animal tends to circle; and spins violently if picked up by the tail.

Five contact experiments were carried out by placing normal mice in the same cage with infected ones. If the infected animals survived, contact was maintained for at least 10 days. Two out of 15 Swiss weanlings, thus exposed, showed otitis media at autopsy. Hemolytic streptococci were recovered in pure culture from the middle ear exudate. The cervical lymph nodes and lungs of these mice were uniformly normal. Five Princeton weanlings, similarly exposed, were normal throughout. In these experiments infection was established by the nasal injection of streptococcus cultures. Two of 3 Princeton weanlings exposed to 3 naturally infected mice developed typical cervical lymphadenitis with positive cultures. In one animal an abscessed lymph node ruptured and the released exudate drained outwards through the perforated skin. One of the originally infected mice, in this series, died and was partially eaten.

Discussion. Group A hemolytic streptococci are tacitly regarded as peculiar to man and the isolation of the present type from naturally infected Princeton mice is an unusual occurrence. The source of this infection is undetermined. Hemolytic streptococci of the usual type have rarely been encountered in mice from the parent colony. The group A

type was not demonstrable in the nasal passages of adults and weanlings obtained directly from the commercial colony. Streptococci were certainly not present in the inocula which the mice received prior to the outbreak of lymphadenitis. Though direct evidence is lacking it may well be that the organisms were originally transmitted to the mice, by way of the nasal passages, from an infected attendant. What effect the preceding experimental treatment may have had on the mice is also uncertain. It is possible that they were more receptive to streptococcal infection than normal mice of the same age. The manifestations of the naturally acquired disease were fully reproduced, however, in the absence of the agents originally employed. Initial establishment of the streptococci in mice of different age groups was probably attended by a limited transmission by direct contact within each cage.

The experimental findings agreed essentially with the earlier results of Sonkin(2), and of Glaser, Berry, and Loeb(3) on the production of murine cervical lymphadenitis by hemolytic streptococci. There was a marked disparity, however, in the death rates resulting from the nasal deposition of the organisms. The susceptibility of the mice and the pathogenicity of the streptococci are variables that must be here considered. The present results were indicative of differences in the response of Princeton and unselected Swiss mice to streptococcal infection. Both of these strains differ genetically from Webster's selected Swiss strain that was used by the earlier workers. The several types of streptococci used in the current and the preceding series vary in their antigenic composition and probably differ in virulence.

Summary. Group A hemolytic streptococci were regularly isolated from the abscessed lymph nodes of Princeton mice during an outbreak of cervical lymphadenitis. The naturally acquired disease was reproduced in normal Princeton weanlings by nasal deposition of the organism in pure culture. The mortality rate was markedly increased, however, by nasal inhalation of the inoculum. In Swiss weanlings the morbidity rate of cervical lymphadenitis after deposition and the mortality rate after inhalation were much reduced. Nasal introduction of the streptococcus was attended by otitis media in 28 out of 45 Princeton wealings (62%) and 40 out of 55 Swiss (72%).

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Protective Action of Tetanus Toxoid Unrelated to Active Immunization in Mice.* (21160)

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Recent studies by us, as well as by others, indicate clearly that an individual previously immunized with tetanus toxoid enjoys a long-continued protection against the action of tetanus toxin, not only in respect of serum antitoxin levels, but in the ability to respond rapidly to a booster dose of toxoid. Even 10 to 11 years after the last immunizing injection, most persons respond to a booster dose of toxoid with a detectable rise in serum antitoxin within 3 to 5 days(1). While the brief lag period in antitoxin response after a booster dose of toxoid probably does not indicate lack of protection, nevertheless it might be useful to find means of supplementing the individual's immunity during this period other than

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