Bacteriologic, Etiologic, and Serologic Studies in Epilepsy and Schizophrenia II.

EFFECTS IN ANIMALS FOLLOWING INOCULATION OF ALPHA STREPTOCOCCI

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The occurrence of convulsions and of psychotic symptoms in low incidence during various infective diseases, some due to neurotropic viruses as now understood, has been considered as presumptive evidence in favor of an infectious etiology of epilepsy and schizophrenia.[15] The possibility that specific types of streptococci might in some way be causative was suggested in experiments on elective localization and specificity of alpha streptococci as isolated in studies of diseases of the nervous system.

Streptococci isolated from patients with infectious peripheral neuritis, when given intravenously to rabbits localized and produced lesions in the peripheral nerves.[1, 2] The streptococci isolated in studies of herpes zoster, a disease now considered to be due to a virus, localized and produced lesions in the posterior columns of the spinal cord and intervertebral ganglia associated with blistering of the skin and great pain characteristic of that disease.[3]

The streptococci isolated in studies of intercostal neuralgia localized in posterior roots of intercostal nerves of rabbits on intravenous injection.[4] The streptococci freshly isolated from a dying pulp of a tooth and from an excised piece of inflamed muscle in a patient suffering from a recurrent severe attack of dental neuritis and myositis localized electively in the pulps of teeth, the dental nerves, and muscles of rabbits and dogs following intravenous injection.[5]

Spasms of the diaphragm were produced consistently on intracerebral inoculation of living cultures, the heat-killed streptococci, and filtrates of active cultures of the streptococcus as isolated in studies of epidemic and persistent postoperative hiccup.[6] Postoperative "ether convulsions" have been reproduced experimentally with a specific type of neurotropic streptococcus.[7] Alpha streptococci freshly isolated from nasopharynx, stool, spinal fluid, and the virus of poliomyelitis on appropriate injection produced flaccid paralysis in guinea pigs, rabbits, and monkeys as the outstanding manifestation,[8] whereas the streptococci similarly isolated in studies of encephalitis[9, 10] produced the symptoms of encephalitis often corresponding in type to the encephalitis in the patient from whom the streptococci was isolated.[11]

The symptoms and lesions characteristic of Sydenham's chorea were produced in rabbits on intracerebral and intravenous injection of the streptococcus isolated from the nasopharynx of patients suffering from Sydenham's chorea, and were produced in dogs through the induction of chronic foci in the teeth with the streptococcus.[12] Spasmodic

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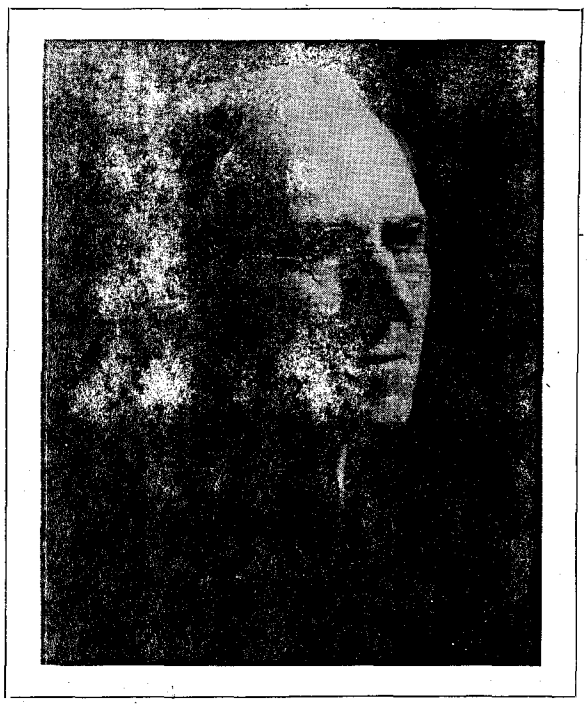
torticollis was produced in high incidence in rabbits,[13] and the strange syndrome of respiratory arrhythmia and mental deterioration following encephalitis was closely simulated[14] on intracerebral injection of the streptococci isolated from the nasopharynx of respective patients.

It should be emphasized that these highly specific results were obtained by the use of special methods. The usual methods did not suffice.[15] Other workers who have used methods essentially like those we have used have reported similar highly specific results in a wide range of diseases, involving tissues derived from both mesoderm and ectoderm. Numerous reports by different investigators deal especially with the question of specificity of alpha streptococci in the causation of disease.*

The concept has been set forth that highly specific types of streptococci may be causative in epilepsy and schizophrenia; for this concept to be valid, proof is needed to show that such specificities may be acquired and that perhaps hereditary predisposition may provide the very conditions favorable for streptococci to acquire the respective specific properties. Experimental evidence is not lacking to show that changes in specificity of alpha streptococci do occur in response to changes in environment. The continual presence of neurotropic and arthrotropic alpha streptococci, respectively, in patients having "neuropathic" or "arthropathic" hereditary predispositions and suffering from chronic encephalitis and chronic arthritis, respectively, has been shown.[31]

Changes in vitro from one type of specificity of alpha streptococci into other types often characteristic of those at hand in, or the cause of, epidemic diseases have been shown to occur seasonally,[32] and have been induced by growth on certain artificial mediums,[1] by successive passage through animals,[33] and by exposing cultures to a high-frequency field of radiant energy.[34] Changes in localization of the freshly isolated streptococci in rabbits following intravenous injection matched changes in localization which occurred spontaneously in patients during successive outbreaks of epidemic hiccup and of neuromyeloencephalitis.[35] The importance of the principles of microbic dissociation in the causation of disease in accord with these experiments has been especially emphasized by Hadley.[36]



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Localization in animals on injection of living cultures of streptococci, the heat-killed organisms, and the toxic components in filtrates of cultures in my studies were often so precise as to resemble the specific pharmacologic action of drugs and chemicals.[37]

Streptococci isolated from the nasopharynx and infected pulpless teeth of persons suffering from epilepsy and schizophrenia have been shown to possess neurotropic distribution curves of cataphoretic velocity.[38] A number of persons who were in the early stages of epilepsy and of psychoneurosis recovered following therapeutic injections of autogenous vaccines prepared from streptococci with neurotropic cataphoretic velocity[38] and one which had produced spasms or mental disorientation, respectively, in rabbits on intracerebral injection.

*Haden,[16] Barnes and Giordano,[17] Nickel and Hufford,[18] Cooper,[19] Harlv and Brinch,[20] Welsh,[21, 22] Meisser and Gardner,[23] Cook,[24] Bernhardt,[25] Irons, Brown, and Nadler,[26] Kelly,[27] Topley and Weir,[28] Wilkie,[29] and Jones and Newsom.[30]

It is the purpose of this paper to describe the methods used, to record unpublished experiments on Macaccus rhesus monkeys which had been inoculated with streptococci from diverse sources, and to report results obtained in a bacteriologic study of epilepsy and schizophrenia and of the localization in animals of the respective streptococci as isolated.

METHODS

Nasopharyngeal swabbings of patients suffering from various diseases, including epilepsy and schizophrenia, were made with aluminum-wire-wrapped swabs bent to a suitable angle so as not to touch the tongue. Pus from tonsils was expressed and scooped with a small bent laryngeal mirror. Purulent material from the depth of pyorrhea pockets was aspirated into capillary pipettes. Severed apexes of pulpless teeth were extracted in a sterile manner. Each of these materials was suspended in 2 ml. of a 0.2 per cent gelatin-Locke or isotonic sodium chloride solution for cultures, precipitation tests, and inoculation of animals.

Cultures were made from spinal fluid, from the centrifugated sediment of freshly voided urine, and from suspensions of stool in sodium chloride solution. The surface of horse blood agar plates was inoculated routinely, and serial dilution cultures[39] were made in mediums affording a gradient of oxygen tension, usually alternately in dextrose-brain broth (0.2 per cent dextrose) and soft dextrose-brain agar (0.2 per cent dextrose and 0.2 per cent agar) in tall (12 cm.) columns in test tubes (⅝ x 6 or 8 inches). The dextrose-brain broth and dextrose-brain agar were usually freshly prepared by adding before autoclaving approximately 1 part by volume of pieces of fresh or frozen calf or young beef brain to approximately 7 volumes, or 15 ml., of the medium, previously adjusted to *p*H 7.2 and then autoclaved at 15 pounds pressure for twenty minutes.

The amount of inoculum in the first tube and the degree of serial dilution was determined roughly by the number and kind of organisms found in Gram-safranine stained films of the material under study. Serial, one hundredfold, dilutions or 10^{-2} of the materials suspended in sodium chloride solution were usually made by transferring 0.15 ml. from tube to tube with a 1 ml. pipette, and 10,000 fold or 10^{-4} dilutions of cultures were made by transferring 1.5 ml. from tube to tube with nichrome wire. The same pipette or wire was used for the serial dilutions without sterilizing between transfers. Hence, the successive dilutions represent dilutions of liquid and not necessarily corresponding dilutions of organisms.

Seven ml. of blood were routinely drawn from the vein at the bend of the elbow into sterile vacuum tubes. The blood was allowed to clot. The rubber stopper was then removed, in a sterile manner, the clot loosened with a sterile capillary pipette, the rubber stopper replaced, and the vial centrifuged. The serum was decanted then, and in a sterile manner, the partially macerated clot was transferred into 200 ml. of freshly prepared dextrose-brain broth. The small amount of blood remaining in the stoppered vial and the inoculated dextrose-brain broth were then incubated. Gramsafranine stained films were made and examined for bacteria as soon as evidence of growth in the broth had occurred, and in five or seven days such films were made from the blood residue in the vial and from the sediment of seemingly negative cultures. All cultures were incubated at 33°C. to 35°C.

Pure cultures of the streptococci for inoculation of animals and other studies were obtained not from primary blood agar plates, but from the end point of growth of usually young primary serial dilution cultures alternately in dextrose-brain broth and dextrose-brain (agar or from young primary cultures in dextrose-brain) broth. Blood agar plates were made of these to determine the type of streptococcus and to check the purity of the cultures.

Monkeys were inoculated intracerebrally usually with 1.5 ml. of the washings of nasopharyngeal swabbings of patients, of corresponding pure cultures in dextrose-brain broth or autoclaved chick embryo medium of the streptococci diluted 1:200 or more, and of emulsions or filtrates of emulsions of the brains and spinal cords of mice, rabbits, or monkeys that had succumbed to poliomyelitis, polio-encephalitis, or encephalitis following intracerebral inoculation. In some instances intraspinus, intravenous, and intramuscular inoculations were given in addition to intracerebral inoculations.

Inoculations were repeated at short or long intervals as indicated by the absence, character, and duration of symptoms and by the nature of the inocula.

Rabbits and mice were inoculated either directly with suspensions in saline or with material obtained from nasopharynx, tonsils, or teeth; with the primary cultures in dextrose-brain broth, with suspensions of single colonies likewise from the end point of growth, or with dextrose-brain broth cultures of freshly isolated strains after a variable number of rapidly repeated subcultures in dextrose-brain broth. Intracerebral inoculations in all instances were made under ether anesthesia.

Routinely, two rabbits were inoculated intracerebrally, one with 0.1 ml., the other with 0.2 ml., of the saline suspensions or with like amounts of 1:200 to 1:10,000, or greater dilution, of the pure cultures of the streptococci in dextrose-brain broth. Mice at first were inoculated intracerebrally with 0.03 ml. of the suspensions and of 1:10 dilution of the pure cultures and intraperitoneally with 1.2 ml. of undiluted cultures. After it was found that largely negative results were obtained as regards the occurrence of characteristic symptoms, a larger number of streptococci was inoculated. Accordingly 0.03 ml. of 10:1 suspensions in sodium chloride solution of young dextrose-brain broth cultures, 20:1 suspensions of the heat-killed organisms, and 10 per cent emulsions of the brains of mice that had succumbed were inoculated intracerebrally, and 0.1 ml. of 20:1 suspensions of dextrose-brain broth cultures of the streptococci were inoculated intranasally. Filtrates of dextrose-brain broth cultures were injected intracerebrally and intravenously in sodium chloride solution in 0.03 ml. and 1 ml. amounts, respectively.

The animals were observed and symptoms recorded at frequent intervals during the day and at crucial points several times during the night. They were examined for lesions under a strong light as soon after death as possible, and cultures were made on blood agar plates and into dextrose-brain broth of brain substance admixed with

cerebrospinal fluid and from the blood from the heart. Pieces of the brain were placed in 10 per cent formalin for sections.

RESULTS OF CULTURES

Cultures on blood agar plates were made of nasopharyngeal swabbings from 181 persons suffering from epilepsy, from 258 persons suffering from schizophrenia, from 85 persons suffering from chronic or subacute arthritis, and as a control from 78 well persons. Green-producing colonies of alpha streptococci grew in predominating numbers in most instances and indifferent colonies grew in a few. Hemolytic or beta streptococci grew in a small number of instances and were never present in predominating numbers. Variable numbers of colonies of *Micrococcus catarrhalis and staphylococci grew in most instances*, and in no instance did Hemophilus influenzae grow in large numbers. There was no distinctive difference between the type of colonies that grew in the different groups. In general, however, the number of colonies was greater, often far greater, in the cultures from persons who were ill than was observed in comparable cultures from well persons.

Shake cultures in blood agar and serial dilution cultures in dextrose-brain broth or alternately in dextrose-brain broth and dextrose-brain agar were made from the spinal fluid in 24, from the urine in 10, from the apexes of pulpless teeth extracted in a sterile manner in 24, and from the stool in 23 persons suffering from schizophrenia. The spinal fluids proved sterile; greening streptococci were isolated in small numbers from the urine and in large numbers from the teeth; and greening or indifferent streptococci were isolated in 19 of the 23 stools cultured.

Cultures in the dextrose-brain broth of the partially macerated blood clot yielded highly pleomorphic greening or indifferent streptococci to blood agar in 49, or 29 per cent, of 161 persons suffering from epilepsy, in only 5, or 4 per cent, of 125 persons suffering from schizophrenia, and in 7, or 14 per cent, of 69 persons having chronic arthritis; the cultures from 62 well persons or persons suffering from noninfectious ailments yielded nothing. The incidence of isolation of streptococci from the blood in persons having epilepsy was highest shortly before or during seizures, and most of the 49 persons from whose blood the streptococcus was isolated were taking phenobarbital or dilantin or both at the time the blood was drawn.

Agglutination tests were made with antiserums prepared in horses and rabbits and with thermal antibodies prepared in vitro with streptococci isolated from nasopharynx or blood in studies of epilepsy, schizophrenia, and arthritis and with the respective serums on the streptococci isolated from the nasopharynx, teeth, blood, urine, and stool. Results of these studies and of the precipitation and intradermal tests with the respective antiserums and thermal antibodies will be reported elsewhere. Suffice it to state here that evidence of respective specificity of the streptococci isolated from the nasopharynx, teeth, and blood was obtained by each of these methods, whereas the strains of streptococci isolated from urine and stool proved nonspecific.

RESULTS OF EXPERIMENTS IN MONKEYS

Convulsions and disorientation of mental processes sometimes occur during or following streptococcal or other infections. In order to determine the conditions under which streptococci and other causative agents may cause spasms or convulsions and disorientation, we reviewed the results of a large number of experiments in monkeys in which such symptoms sometime developed following inoculation of (1) natural or experimentally produced poliomyelitic and encephalitic virus, (2) of streptococci isolated from these and from nasopharynx and spinal fluid of patients and monkeys that had succumbed to poliomyelitis, polioencephalitis, or encephalitis, and (3) of streptococci isolated from nature in relation to epidemics of poliomyelitis and encephalitis and in relation to nonepidemic diseases.

Of a total of 1,338 monkeys that were inoculated with cultures of streptococci or material containing streptococci during the years 1917 to 1944, spasms or convulsions were recorded to have occurred in 109, or 8 per cent, and symptoms simulating in certain respects those of schizophrenia in 24, or 1.8 per cent. Table 1 summarizes the incidence of spasms or convulsions that developed in the Macaccus monkeys following inoculation of material containing streptococci in relation to poliomyelitis and encephalitis and the incidence of the demonstration in, or the isolation of, streptococci from spinal fluid or brain of animals in which spasms developed.*

It will be seen that the incidence of spasms or convulsions and the demonstration of streptococci in spinal fluid or brain in the monkeys in which spasms had developed, in general, paralleled the incidence of spasms or convulsions and the ease with which streptococci are demonstrable in the respective spontaneous diseases. Moreover, the incidence, severity, and duration of spasms of convulsions in the different groups of inoculated monkeys paralleled roughly the incidence of spasms which occur in spontaneous poliomyelitis, polioencephalitis, and encephalitis.

The drop in incidence of spasms from 26, or 54 per cent, of the 48 monkeys that received the experimental streptococcal virus while in the encephalitic phase to 3, or 6 per cent, of 51 monkeys that received the experimental virus in the poliomyelitic phase is especially noteworthy.[40, 41, 42] Of 109 monkeys developing spasms, 36 of them received Berkefeld or Seitz filtrates of brain or cord emulsions of animals that had been inoculated with virus containing streptococci or with filtrates of chick embryo cultures of streptococci; 48 received emulsions of the brains of mice, rabbits, or monkeys that had been inoculated with natural or experimentally produced virus and from which streptococci were isolated; and 25 received cultures of neurotropic alpha streptococci in high dilution.

The onset of spasms ranged from two to sixteen days after inoculation in the different groups and averaged 5.2 days. In most instances the spasms resembled those obtained following inoculation of the streptococcus isolated

*The experiments on monkeys and on some of the rabbits were done at the Mayo Foundation for Medical Education and Research, Rochester, Minnesota, during the course of studies on the relation of the streptococcus to poliomyelitis and encephalitis and their respective viruses.

TABLE 1

INCIDENCE OF SPASMS OR CONVULSIONS IN MACACCUS RHESUS MONKEYS FOLLOWING INOCULATION OF NATURAL AND EXPERIMENTAL POLIOMYELITIC AND ENCEPHALITIC VIRUS AND OF NEUROTROPIC ALPHA STREPTOCOCCI ISOLATED IN STUDIES OF POLIOMYELITIS AND ENCEPHALITIS

Material inoculated		Strains	Monkeys inoculated	Monkeys in which spasms or convulsions developed: Number	Per cent	And in which the streptococcus was demonstrated in spinal fluid or brain: Number examined	Yielding streptococci: Number	Per cent
Natural poliomyelitic virus		12	214	17	8	12	5	42
Natural encephalitic virus		17	97	18	19	13	8	62
Experimental streptococcal virus in:	Encephalitic phase	6	48	26	54	22	12	55
	Poliomyelitic phase	6	51	3	6	3	1	33
Alpha streptococci isolated from nasopharynx, spinal fluid, or brain and spinal cord of persons and monkeys that had poliomyelitis or encephalitis		23	162	25	15	20	15	75
Alpha streptococci isolated from milk and water supplies, from air, flies, and bedbugs (Cemex lectularius) in relation to poliomyelitis and encephalitis		20	57	20	35	13	9	69
Total		80	629	109	17	83	50	60

in studies of myoclonic encephalitis.[11] In a few instances the character of the spasms resembled petit mal and grand mal of epilepsy, well illustrated in Protocol 1 later in this paper. The results obtained in monkeys inoculated with the streptococcus isolated in studies of epilepsy are depicted in Protocol 2.

The strange behavior, such as the threatening to attack, the extreme excitation in which the animal dashed about violently, the apparent seeing of imaginary objects or hearing of noises, and cataleptic states, occurred as an integral part of other symptoms, such as blurred vision or blindness, nystagmus, myoclonic spasms, and flaccid or spastic paresis or paralysis (Protocol 3). Aside from a variable congestion of the brain of the monkeys in which spasms or disorientation had developed, little was found at necropsy. Abscess and grossly visible meningitis were absent. Microscopic sections in general revealed the lesions characteristic of the respective experimental disease in question.

During the course of these studies, I had opportunity to inoculate monkeys and rabbits with streptococci isolated from the nasopharynx of a patient, a middle-aged woman, who was suffering from an acute attack of "psychoneurosis." The patient made a slow recovery under insulin shock treatment. She remained apparently well for eight years, when she had a recurrence incident to the death of a son.

My studies were made at the time of an attempted suicide during her first attack. Material obtained on the swab from her nasopharynx was washed off in 2 ml. of the sodium chloride solution. Blood agar streak cultures of the sodium chloride suspension revealed large numbers of colonies of alpha streptococci, and short-chained streptococci grew out in pure culture in dextrose-brain broth. The sodium chloride solution suspension of the nasopharyngeal swabbing was inoculated intracerebrally into the right frontal lobe of two rabbits and one monkey.

Severe tremors and excitation developed in both rabbits. At times they dashed about in a wild fashion unmolested in their cages. Hemorrhagic edema of the lungs and severe congestion of the brain were found at necropsy in both rabbits, and large numbers of the streptococci were isolated from the brain and blood after death.

Symptoms in the monkey were similar. It became extremely excitable the day after inoculation. On the second day, and for five days thereafter, excitability and hyperirritability were extreme. The animal seemed disoriented most of the time. It often acted as if it saw imaginary threatening objects always on its left side. Spinal puncture made on the day following inoculation revealed slightly turbid fluid from which the streptococcus was isolated in pure culture. The brain after death was diffusely congested. There was no gross evidence of meningitis and no mark at the site of intracerebral inoculation in the right frontal lobe. A large blood clot was found in the left cerebral ventricle adherent to the choroid plexus. The viscera were normal. Cultures in dextrose-brain broth of pipettings of the brain substance admixed with cerebrospinal fluid yielded a pure culture of the streptococcus. Cultures from the blood and brain on blood agar plates proved sterile.

One additional rabbit was inoculated with 1.2 ml. of a 1:200 dilution of the dextrose-brain broth culture of the streptococcus isolated from the spinal fluid on the

second day, and one monkey was given 2 ml. intracerebrally and 3 ml. intraspinously of a Berkefeld filtrate of 10 per cent emulsion of the brain of the monkey. The third rabbit developed symptoms similar to those shown by the two rabbits that received the sodium chloride solution suspension from the nasopharynx of the patient. The monkey remained free from symptoms for twenty days, when it became extremely excitable and repeatedly threatened to charge when observed unmolested in its cage. On being prodded it developed severe tremors and slight clonic spasms. The temperature was 106°F. The following day the temperature was 105.2°F. It continued to be extremely excitable, threatening to charge for several days; then it recovered gradually. It remained well for three months, when it was inoculated with material from another source.

EXPERIMENTS IN RABBITS WITH STREPTOCOCCI ISOLATED IN STUDIES OF EPILEPSY AND SCHIZOPHRENIA

The occurrence at times of spasms or convulsions and of disorientation in monkeys following inoculation of streptococci or material containing streptococci from diverse sources led to a study of the effects in rabbits of streptococci isolated in studies of epilepsy and schizophrenia. The results obtained in rabbits are summarized in Table 2. When rabbits were inoculated with the living cultures of the streptococcus, the dead bacteria, and filtrates of cultures from the nasopharynx and blood of persons suffering from epilepsy, the incidence of hyperirritability was far less, the incidence of tremors was about the same, and the incidence of spasms and convulsions was far greater than when they were given identical inoculations of the streptococcus isolated from the nasopharynx of persons suffering from schizophrenia. The mortality and isolations of streptococci from the brains of rabbits that succumbed was about the same in the two groups, but the isolations of streptococci from the blood following inoculation of the streptococcus from epilepsy was from three to five times as great in the different groups that received living streptococci as following inoculation of the streptococcus from schizophrenia.

The source and type of inocula, the number and type of streptococci, and the time of onset of symptoms were all comparable. But as symptoms progressed, a striking difference became apparent. Severe tremors and spasms of masseters, generalized tremors and ataxia, clonic and tonic spasms, and often recurring generalized convulsions resembling grand mal developed in rabbits that received the living or dead streptococci or the filtrate of cultures isolated from the nasopharynx or blood of persons suffering from epilepsy (Protocols 4 and 5). In sharp contrast extreme hyperirritability with evidence of disorientation usually without spasms developed in the rabbits inoculated with the streptococcus isolated from the nasopharynx of persons suffering from schizophrenia or manic-depressive psychosis (Protocol 6).

Symptoms characteristic of the disease in question usually developed in a large percentage of animals inoculated with the streptococcus of a given case, especially if cultures were obtained at the time of acute attacks. Myoclonic spasms developed in each of a group of rabbits inoculated respectively with the streptococcus isolated from the nasopharynx, blood, and an extracted pulpless tooth of a man, a teacher who was becoming incapacitated because of recurring attacks of grand mal. Following the extraction of the tooth, his attacks of grand mal disappeared and had not recurred for nine years.

In sharp contrast, severe tremors and extreme hyperirritability sometimes accompanied by mild clonic spasms developed in each of 11 rabbits following intracerebral inoculation of a highly diluted culture of the streptococcus isolated, shortly after death, from the nasopharynx of a young woman who committed suicide by jumping from the top of a tall building during an acute attack of "psychoneurosis." The streptococcus inoculated was so far removed from the original source as to eliminate the possibility of the presence of virus.

RESULTS OF EXPERIMENTS IN MICE

At first mice were inoculated intracerebrally with 0.03 ml. of the sodium chloride solution suspension or with the primary young culture of the streptococcus undiluted or diluted 1:10 in sodium chloride solution of nasopharyngeal swabbings of persons suffering from epilepsy or schizophrenia. The results were considered as suggestive of specificity of the respective streptococci.

Thus, 8, or 18 per cent, of 43 mice that received the streptococcus from 35 persons suffering from epilepsy were seen to have severe tremors, twitchings, and mild clonic spasms, and 68, or 33 per cent, of 205 mice similarly inoculated with the streptococcus from the nasopharynx of 55 persons who were suffering from schizophrenia developed hyperirritability and, more rarely, catatonic states that were usually without spasms.

Since mice proved relatively resistant to the streptococcus, it was thought that if larger numbers of the streptococci were inoculated, especially streptococci obtained from the end point of growth in serial dilution cultures of dextrose-brain broth, a higher incidence of respective specific effects might be obtained. Accordingly, we inoculated intracerebrally 0.03 ml. of 10:1 suspensions in saline of dextrose-brain broth cultures and suspensions of similar density of the streptococci from streak cultures on blood agar plates made with the brains of mice that succumbed to inoculations.

In the experiments summarized in Table 3, two pooled suspensions of streptococci from cultures at the end point of growth of the nasopharyngeal swabbings—one from 13 and the other from 11 persons suffering from severe epilepsy—and corresponding cultures from each of 5 other persons having epilepsy, or seven suspensions in all, were inoculated into 17 mice in the first animal passage; 4 strains were used in the second passage; and then further animal passages were made with the strain derived from pooled cultures from the 11 cases. Spasms occurred in the mice following the inoculation of each of the suspensions of the different pools and strains. The cultures from the nasopharynx of well persons used as a control group were similarly pooled and similarly inoculated. Spasms developed in only 3 of 23 mice in the first passage, and in none of 21 mice in the second passage; hence, no further animal passage of control strains were done.

It will be seen that the incidence of spasms and convulsions following intracerebral inoculation of the living

TABLE 2

MORTALITY, SYMPTOMS AND SIGNS, AND ISOLATION OF STREPTOCOCCI FROM THE BRAIN AND BLOOD OF RABBITS FOLLOWING INTRACEREBRAL INOCULATION OF STREPTOCOCCI ISOLATED FROM PERSONS SUFFERING FROM EPILEPSY AND SCHIZOPHRENIA

Source of streptococci	Material injected intracerebrally	Strains or cases	Rabbits		Symptoms or findings in per cent				Streptococci			
									Brain		Blood	
			In-jected	Per cent died	Hyper-irrita-bility	Tre-mors	Spasms	Convul-sions	Cul-tured	Per cent	Cul-tured	Per cent
Epilepsy (46 Cases)	Suspension of material directly from naso-pharynx, tonsils, or teeth	37	72	65	29	**75**	**75**	**33**	47	94	46	26
	Dextrose-brain broth cultures from naso-pharynx, tonsils, teeth, or blood diluted 1:200 to 1:10,000	26	34	91	15	74	73	**35**	25	92	23	43
	Total for material containing living strepto-cocci	63	106	74	25	**75**	**75**	**34**	72	93	69	32
	Heat killed streptococci and filtrates of dextrose-brain broth cultures	10	19	58	11	**74**	**79**	**47**	11	0	6	0
Schizophrenia (45 Cases)	Suspensions of material directly from naso-pharynx or dextrose-brain broth cultures diluted 1:200 to 1:10,000	45	77	87	**87**	**79**	21	3	54	83	46	7

streptococcus obtained from persons suffering from epilepsy was remarkably high throughout nine successive passages, averaging 93 per cent and 69 per cent, respectively, and that spasms and convulsions almost never occurred in control mice inoculated in identical manner with the streptococcus isolated from the control group of well persons. The incidence of spasms and convulsions following inoculation of suspensions of the heat-killed streptococci and filtrates of dextrose-brain broth cultures of streptococci isolated from persons having epilepsy was also high, whereas no spasms developed in mice that received suspensions and filtrates from the control group.

The statistical evidence of specificity shown in Table 3, though striking, does not express adequately the specific character and type of symptoms that developed in these mice following inoculation of the living and the heat-killed streptococcus and filtrates of cultures.

The mice usually remained apparently well for from four to twelve, and sometimes for twenty-four, hours after inoculation. Evidence of illness was first manifested by their becoming abnormally quiet and standing humped up with roughened fur and by increased respiration and fine and coarse tremors; these were followed somewhat later by hyperirritability, and then in rapid succession by scratching of the nose or side of the head, severe tremors, twitching, and clonic spasms of muscles of the face, ears, neck, and extremities. They then fell to the side as the head was pulled violently to one side or other or backward, as generalized tonic followed by clonic spasms and often voiding of urine occurred. These symptoms were followed in turn by increased respiration as they lay relaxed, apparently unconscious and insensitive for a moment to stimuli or proddings. Recovery often appeared to be complete as symptoms developed soon after inoculation, but as symptoms became worse hour by hour or day by day, the spasms and convulsions were almost continuous, and in most instances the mice died apparently from exhaustion during seizures resembling "status epilepticus."

Of the 130 mice inoculated with the streptococcus from the nasopharynx of persons suffering from severe epilepsy, 102, or 78 per cent, died in from one to eight days. Spasms did not occur at all in 12 of the 28 that survived, and the recurring seizures in the remaining 16 disappeared during the course of two weeks.

Of the 44 mice inoculated with the streptococcus from the nasopharynx of well persons used as a control, 12, or 27 per cent, died. Seven of these died within twenty-four hours, and 5 died on the second day after inoculation.

The tendency of the strains from persons having epilepsy to produce spasms and convulsions when grown in dextrose-brain broth persisted through consecutive serial dilution cultures representing dilutions of original inoculum of at least 10^{-10} to 10^{-80}, and as shown in Table 3, persisted through nine consecutive passages through mice following inoculation of brain emulsions, primary cultures, and of from three to five subcultures rapidly repeated. The spasm-producing property was found to be present in undiminished form in one strain that had been passed through six series of mice on isolation after it was kept at room temperature dried on glass balls in vacuo under a dessicant and on a sealed blood agar slant for three weeks and after seven rapidly repeated (two or three per day) subcultures in dextrose-brain broth. It was

then subcultured in dextrose-brain broth every third or fourth day for five additional subcultures, whereupon it had not only lost the power to produce spasms but apparently all virulence, since spasms did not develop in any of the 10 mice, 2 rabbits, and 2 rats inoculated, and all animals survived.

Having found a fairly precise method for measuring symptom-producing and localizing power of streptococci in mice, we studied the comparative effect on mice of the intracerebral and intranasal inoculation of streptococci isolated from the nasopharynx of persons suffering from different types of epilepsy, persons suffering from dementia paralytica with and without convulsions, of well persons; and also the inoculation of streptococci isolated from the brain of an uninoculated baby mouse that died in convulsions, and of streptococci isolated from poliomyelitic virus virulent for monkey and mice.*

The mother of the baby mouse that was seen to die in spasms had been inoculated intranasally several times, in the early stages of gestation, with the streptococcus from a person suffering from grand mal epilepsy in the seventh mouse passage. No symptoms were noted either in the mother mouse or in the three litter mates which grew to maturity without spasms or convulsions.

The streptococci from the end point of growth of the usual serial dilution cultures were inoculated intracerebrally under ether anesthesia in the usual and comparable dosage for the different strains. Intranasal inoculations were made under deep ether anesthesia with 20:1 suspensions in sodium chloride solution of the respective streptococci. These were usually repeated once or twice daily for three or four times. An attempt was made to lower the inherently high resistance of the brain to invasion of noxious agents, by injecting 0.03 ml. of sterile sodium chloride solution intracerebrally just before or after the first intranasal inoculation in alternate mice and in control mice. No symptoms developed in the latter group. The number of "takes" in mice that received sodium chloride solution intracerebrally and the streptococcus intranasally was approximately twice as great as in those that received only intranasal inoculations. The results in this series of experiments are summarized in Table 4.

*I am indebted to Dr. C. A. Armstrong, Washington, D. C., for the poliomyelitic virus virulent for monkey and for the Lansing strain of virus adapted to the mouse and to C. W. Jungeblut, New York, for two strains of murine poliomyelitic virus.

IT WILL be seen that (1) the incidence of spasms and convulsions was uniformly much higher in mice that received intracerebral inoculations of the streptococci isolated from conditions characterized by spasms or convulsions (epilepsy, dementia paralytica, and the mouse with convulsions)—spasms occurred in 81, or 88 per cent, and generalized convulsions in 64, or 70 per cent, of 92 mice so inoculated—than in those that received inoculations from conditions in which spasms or convulsions were absent— spasms occurring in 10, or 11 per cent, and convulsions in 2, or 2 per cent, of 89 mice so inoculated; (2) spasms and convulsions occurred in significant incidence following intranasal inoculation of the streptococci isolated in studies of each of the three types of epilepsy; (3) paralysis occurred in 24, or 45 per cent, of 53 mice that

TABLE 3

RESULTS IN MICE FOLLOWING INTRACEREBRAL INOCULATION OF STREPTOCOCCI ISOLATED FROM THE NASOPHARYNX OF PERSONS SUFFERING FROM IDIOPATHIC EPILEPSY AND FROM WELL CONTROLS

Source of streptococci		Animal passage	Strains or Cases	Mice: Inoculated	Mice: Seen to have: Spasms	Mice: Seen to have: Convulsions	Mice: Died	Mice: Cultured	Streptococcus from: Brain	Streptococcus from: Blood
Nasopharynx of persons suffering from idiopathic epilepsy		1	29	17	16	10	20	11	11	6
		2	4	18	16	11	14	10	10	5
		3	1	9	9	7	8	7	5	2
		4	1	6	5	3	7	4	4	2
		5	1	39	38	33	27	24	25	18
		6	1	14	14	11	11	10	9	7
		7	1	15	12	9	10	5	4	3
		8	1	8	8	4	7	7	7	7
		9	1	4	3	2	3	3	3	1
Total		1–9	30	130	121 (93%)	90 (69%)	107 (82%)	81	78 (98%)	51 (63%)
Nasopharynx of control well persons		1	32	23	3	1	9	8	7	4
		2	8	21	0	0	3	1	1	1
Total		1–2	32	44	3 (7%)	1 (2%)	12 (28%)	9	8 (89%)	5 (56%)
Epilepsy	Heat killed streptococci		4	15	11 (73%)	9 (60%)	8 (53%)	8	0	0
Control			4	12	0	0	0	0	0	0
Epilepsy	Filtrate of cultures of streptococci		6	22	11 (50%)	4 (18%)	2 (9%)	4	0	0
Control	Filtrate of uninoculated broth		5	10	0	0	0	0	0	0

TABLE 4

RESULTS IN MICE FOLLOWING INOCULATION OF STREPTOCOCCI ISOLATED FROM THE NASOPHARYNX OF PERSONS SUFFERING FROM DIFFERENT TYPES OF EPILEPSY, OF WELL PERSONS AND OF PERSONS SUFFERING FROM DEMENTIA PARALYTICA WITH AND WITHOUT CONVULSIONS, FROM THE BRAIN OF A BABY MOUSE THAT DIED IN CONVULSIONS, AND FROM THE BRAIN AND SPINAL CORD OF MONKEY AND MICE THAT HAD SUCCUMBED TO "VIRUS" POLIOMYELITIS.

Source of streptococci	Animal passage	Strains or Cases	Inoculation	Mice						
				Inoculated	Seen to have: Spasms	Convulsions	Paralysis	Died	Cultured	Streptococci from brain
"Grand mal" epilepsy	6 and 7	1	Cerebral	20	16	12	0	15	9	8
			Nasal	11	6	3	0	3	3	2
"Grand mal" epilepsy	1 and 2	1	Cerebral	8	8	7	0	8	3	3
			Nasal	11	5	1	0	2	1	0
"Petit mal" epilepsy	1 and 2	1	Cerebral	9	9	7	0	7	7	6
			Nasal	17	5	3	0	3	3	0
Mentally deteriorated epileptic	1 and 2	1	Cerebral	14	11	10	0	11	8	5
			Nasal	11	6	2	0	1	1	0
Total for cases of epilepsy	1, 2, 6, and 7	4	Cerebral	51	**44**	**36**	0	41	27	22
			Nasal	50	**22**	**9**	0	9	8	2
Dementia paralytica: With convulsions	1 and 2	6		19	**18**	**12**	0	12	5	5
Dementia paralytica: Without convulsions	1	6		18	3	1	0	14	5	5
Control, well persons	1	6	Cerebral	18	3	0	0	11	6	6
Baby mouse (9 grams) that died in convulsions	1 and 2	1		22	**19**	**16**	0	17	11	10
Poliomyelitic virus	1 to 5	4		53	4	1	**24**	36	25	21

were inoculated with the four strains of streptococci isolated from the brain and spinal cord of a monkey and of mice that died of poliomyelitis following inoculation of virus; this paralysis did not occur in any of 110 mice similarly inoculated with streptococci from sources in which paralysis was absent; and (4) cultures of the brain of 8 mice that developed spasms and that died following intranasal inoculation of the streptococcus from epileptics yielded the streptococcus in only two instances, indicating that the spasms were due perhaps to the absorption of specific neurotoxin from the nasopharynx and from the site of inoculation; the streptococcus was isolated from these areas after death in each instance.

Frothing at the mouth during convulsive seizures was not observed in either mice or rabbits, but sometimes occurred in monkeys during severe convulsive seizures resembling grand mal. Symptoms resembling petit mal and psychic seizures of epilepsy seemingly occurred in mice, rabbits, and monkeys after they were inoculated with the living and heat-killed streptococcus isolated from nasopharynx and blood of persons suffering from epilepsy.

The brain after death in monkeys and rabbits that had succumbed during acute symptoms and in mice that died after spasms had disappeared usually revealed severe congestion, whereas the brain, especially the cerebral cortex, of the mice that succumbed during "status epilepticus" was ischemic, edematous, and usually showed severe cloudy swelling. In no instance was there hemorrhage or abscess formation at the site of inoculation, and grossly visible meningitis was seen in only one mouse in which cultures revealed contamination with E. coli. There were no noteworthy lesions of the viscera.

Sections of the brains of monkeys, rabbits, and mice stained by hematoxylin and eosin, by the Morgan iron hematoxylin method, and for bacteria by a modification of the Gram-Weigert method in which decolorization with alcohol was carried only to a fair blue instead of to the end point, have been examined for lesions and for bacteria.

Those from animals that received inoculations of streptococci isolated in studies of schizophrenia showed only slight or moderate congestion of meninges and cerebral cortex and only slight, widely scattered areas of cellular infiltration. Those from monkeys and rabbits that had been inoculated with streptococci isolated in studies of epilepsy revealed marked congestion and moderate infiltration by leukocytes of meninges in sulci and in superficial layers of the cortex. Sections of the brain of mice that were inoculated with the streptococci from epileptics revealed only slight infiltration of the meninges and the superficial layers of the cerebral cortex, but showed moderate infiltration of the subcortex and severe infiltrations by leukocytes surrounding the choroid plexus and in localized areas of necrosis in the walls of the ventricles. Large numbers of streptococci, chiefly within leukocytes, were found in sections of the brain of animals that died in "status epilepticus" soon after inoculation of streptococci, and only a few or none were demonstrable in animals that died or were anesthetized long after inoculation of living cultures or that died of spasms soon after inoculation of filtrates of cultures. Perivascular infiltration by lymphocytes characteristic of encephalitis was not found regardless of the species of animal or the duration of experiment following inoculation.

The occurrence of maximal lesions, including necrosis, in the walls of the cerebral ventricles in the brain of mice that succumbed to epileptiform seizures following inoculation of the streptococcus isolated from the nasopharynx or blood of persons suffering from epilepsy are in accord with the changes found in the region of the tuber cinereum in the brain of persons suffering from idiopathic epilepsy by Morgan[44] and by Morgan and Gregory[45] and those induced by Morgan and Johnson[46] in the tuber cinereum in the brain of dogs in which epileptiform seizures developed.

ILLUSTRATIVE PROTOCOLS

Protocol 1—A small Macaccus rhesus monkey was inoculated intracerebrally in the right frontal lobe January 7, 1935, with 1.5 ml. of the emulsion of the brain of a mouse that had succumbed to symptoms of polioencephalitis after inoculation of a streptococcus isolated from a water supply of a case of polioencephalitis. The streptococcus, before inoculation into the mouse, was in the tenth rapidly repeated subculture in dextrose-brain broth. Cultures from emulsions of the brain yielded a pure growth of alpha streptococci.

January 9: The animal was excitable, ataxic, and tremulous, and had diminution of vision. The pupils were dilated, and there was slight weakness of the left fore extremity. The condition remained about the same for nine days, when a violent generalized convulsion occurred in which the animal lost its balance, voided urine, and lay unconscious for five minutes; consciousness gradually returned and the animal appeared to be recovered.

February 13: Twenty-six days after inoculation, the monkey was anesthetized and inoculated intracerebrally with 2 ml. of a 10 per cent emulsion of the brains of 3 mice that had succumbed with symptoms of encephalitis after being inoculated with an experimental streptococcal virus strain while in the encephalitic phase.[40]

February 15: The animal was very excitable, ataxic, and tremulous. Its vision was impaired. There was partial paralysis of the right fore and hind extremities. There was little change in the animal's condition until March 5, when it again had a severe generalized convulsion with urination and loss of consciousness resembling a grand mal seizure.

March 6: The monkey was excitable and ataxic; it missed its mark badly on jumping out of and into its cage because of blurred vision and incoordination. It had another violent epileptiform seizure with generalized tonic spasms followed by clonic spasms, frothing at the mouth, urination, and unconsciousness.

March 9: The animal repeatedly had spells apparently of momentary loss of consciousness, in which it sometimes fell to the floor without spasms; these spells resembled petit mal.

March 10 and 11: Ataxia and hyperirritability continued, resembling grand and petit mal.

March 12: The monkey was reinoculated with the emulsion of the brains of 2 mice that had succumbed to two further passages of the same virus diluted three hundredfold in sodium chloride solution.

March 13: The animal was ataxic and hyperirritable, and on jumping from its cage it had a severe generalized convulsion resembling grand mal.

March 18: The animal seemed better, and no convulsions were noted for eleven days.

March 29: The animal was inoculated with a 10 per cent emulsion diluted one thousandfold of the brains of 2 other mice that had succumbed to further successive passages of the experimental virus. The animal remained about the same, and no spasms were noted until April 8, at which time a violent seizure recurred.

April 9: The monkey was less irritable; however, it missed its mark badly in jumping out of and into its cage, and when observed in its cage it had "lapses" resembling petit mal.

April 12: There was a recurrence of hyperirritability. The animal was more ataxic; generalized spasms did not occur, but it had severe tremors of its head associated with mild clonic spasms. The temperature was 104.8°F.

April 13: There was now great weakness of hind extremities and ptosis of the eyelids; the temperature was 103.4°F.

April 14: The tremors continued, but no spasms were noted. The animal was lethargic, and great weakness of hind extremities had developed. The temperature was 97°F.

April 15: The extremities were spastic, and they were so weak that the animal could not stand.

April 16: Condition was about the same, but on prodding mild generalized spasms developed. The temperature was 98° F. The animal was anesthetized to death with ether. There was moderate congestion of the cerebral cortex. The cerebrospinal fluid was greatly increased in amount but was clear. The cerebral ventricles were dilated. There was a small cyst at the site of intracerebral inoculation. Cultures from the pipetting of the brain proved sterile.

Protocol 2—A medium-sized Macaccus rhesus monkey was inoculated intracerebrally December 20, 1936, with 1 ml. of a 1:200 dilution of a dextrose-brain broth culture of a streptococcus isolated from the blood of a patient who suffered from nocturnal epilepsy.

The animal remained well until December 25, when severe tremors and weakness of the hind extremities developed.

December 26: The animal had recurring seizures of generalized tonic and clonic spasms in which it lost its balance and fell to the floor; in severe seizures it voided urine, sometimes frothed at the mouth, and became unconscious for five minutes or more after which consciousness returned, and spasms disappeared. The attacks of spasms recurred at frequent intervals but in progressively milder form for ten days; they then disappeared as the animal recovered. It was observed until January 29, 1937, and no additional spasms were noted.

Protocol 3—A medium-sized Macaccus rhesus monkey was inoculated May 20, 1935, with the Berkefeld filtrate of a 5 per cent emulsion of the brain and spinal cord of a monkey that had succumbed to an intracerebral inoculation of experimental virus derived from a hemolytic streptococcus originally isolated from a case of endocarditis and which had been changed into an alpha streptococcus having neurotropic cataphoretic velocity and virulence and from which a filtrable virus was produced.[40] The experimental virus used for inoculation had been passed consecutively through 8 series of mice nearly all of which developed encephalitis. Cultures from the filtrate inoculated proved sterile. The monkey's temperature was 101°F. Of the filtrate 1.5 ml. was injected intracerebrally, 2 ml. intraspinally, 10 ml. intravenously, and 10 ml. intraperitoneally.

May 23: Cultures from the spinal fluid obtained at the time of inoculation were sterile. Gram-safranine stains of the centrifugated sediment of the spinal fluid revealed lymphocytes and a few gram-positive diplococci, and a short-chained streptococcus was isolated in dextrose-brain broth. The animal was fussy, and its temperature was 104°F. The injections were repeated.

May 24: The animal was hyperirritable, and the face was flushed. The temperature was 104.6°F.

May 26: The animal appeared abnormally quiet and half-dazed. The temperature was 101.6°F.

May 27: The animal acted strangely and kept looking to the right seemingly at imaginary objects; when prodded it suddenly seemed to realize its surroundings and became excitable and tremulous after which its strange action returned. Spinal puncture revealed slightly turbid spinal fluid due to lymphocytes, and in the centrifuged stained sediment a few unmistakable gram-positive diplococci were found. The temperature was 104°F.

May 28: 8 A.M.—Most of the time the animal did not seem to realize what was going on and still kept looking wildly about at imaginary objects. 6 P.M.—The animal was semicoma-

tose, in a generally rigid state resembling catalepsy, and had developed marked weakness, especially of the fore extremities. Respirations were slow, and heart action was very rapid. The temperature was 100.8°F. Spinal puncture revealed clear fluid and no bacteria.

May 29: Cataleptic symptoms were less marked. The animal lay on its side alternately in relaxed and rigid states. There was continual vertical nystagmus and marked drooling of saliva. It had repeated mild generalized clonic spasms in which the animal opened its mouth widely as spasms of the muscles of the jaw and tongue occurred. The animal was anesthetized to death. There was severe congestion of the brain; a small cyst was found in the right frontal lobe at the point of intracerebral injection. Cultures from pipettings of brain substance admixed with spinal fluid proved sterile. Sections of the brain revealed mild perivascular and parenchymatous round cell infiltration.

Protocol 4—A large rabbit weighing 2,850 gm. was inoculated intracerebrally January 27, 1936, with 1 ml. of the saline suspension of the nasopharyngeal swabbing of a patient suffering from severe epileptic seizures.

January 28: 8 A.M.—Tremors of masseters and mild clonic spasms of the muscles of the face and ears were noted. 9 P.M.—The animal was ataxic, respirations were greatly increased, weakness of hind extremities had developed, and mild generalized clonic spasms occurred at intervals.

January 29: 8 A.M.—The tremors were more marked and clonic spasms occurred frequently. 11:30 A.M.—A generalized spasm resembling grand mal occurred. 2 P.M.—While being watched, the animal suddenly leaped forward, fell to its side in violent tonic spasm followed by severe generalized clonic spasms. It voided urine and then lay unconscious with stertorous respiration for a short time. During this period it did not respond to stimuli. As consciousness returned, the animal rose to its feet; it was then free from spasms for two hours, when suddenly a similar but more violet seizure occurred, during which it died in generalized tonic spasms from respiratory failure. Necropsy revealed severe congestion of the brain, slight clouding of meninges, no mark at the site of intracerebral injection, and no lesions of the viscera. The streptococcus was isolated from the brain. The blood proved sterile.

Protocol 5—A white rabbit weighing 2,000 gm. was inoculated intracerebrally June 1, 1935, at 10 A.M. with 0.5 ml. of a sodium chloride solution suspension of the heat-killed streptococcus isolated from the nasopharynx of a patient suffering from severe epilepsy. At 2 P.M. and 5 P.M. marked tremors of masseters were elicited by palpation. At 8 P.M. the animal, while sitting quietly in cage, suddenly cried out as it developed a violent convulsive seizure. Spasms were tonic at first, then clonic as it apparently lost consciousness and stertorous, extremely irregular respiration developed. Shortly following this the animal regained consciousness and was again free from spasms; tremors of masseters continued, however.

June 2: 9 A.M.—The respirations were rapid; the animal was ataxic and tremulous, and tremors of masseters continued. 12 M.—The animal again developed a violent convulsion and died of respiratory failure with head retracted and extremities rigidly outstretched. Necropsy revealed severe congestion of the cerebral cortex and no mark at the site of injection. Cultures from brain and blood were sterile.

Protocol 6—A white and gray rabbit weighing 2,000 gm. was inoculated intracerebrally August 28, 1936, with 0.1 ml. of a culture of the streptococcus in autoclaved chick embryo medium isolated from the nasopharynx of a patient suffering from manic-depressive psychosis.

August 29, 30, and 31: The animal was extremely excitable and tremulous. It apparently imagined that it was about to be attacked since it repeatedly jumped violently about in its cage, and unknowingly jumped out of the cage when the door was opened. During quiet intervals the waving of one's hand over its body caused it to jump violently about.

September 1: The animal was greatly improved. It was anesthetized with ether and reinoculated. Following this inoculation, it again became hyperirritable and instead of being frightened by imaginary objects it became extremely cross and it repeatedly threatened to charge.

September 3: The injection was repeated. The symptoms persisted, and the animal was found dead September 5. Necropsy revealed moderate congestion of the brain, no meningitis, no mark at the site of intracerebral inoculation, and no lesions of the viscera. The streptococcus was isolated from the brain in dextrose-brain broth. The blood proved sterile.

Protocol 7—A white mouse weighing 30 gm. was inoculated intracerebrally June 6, 1946, at 8 A.M. with 0.03 ml. of a 10:1 sodium chloride solution suspension of a streptococcus from a dextrose-brain broth culture of the nasopharynx of a patient suffering from idiopathic epilepsy and from the brain of a mouse that had succumbed to convulsive seizures following inoculation of this strain in the fifth mouse passage. The streptococcus before inoculation into the mouse was derived from growth in extremely high dilution and was in the second subculture. At 8 P.M. slight tremors had developed and respirations increased. At 9:35 P.M. its fur was ruffled and tremors were more pronounced. While it was being observed, recurring tonic and clonic spasms developed, beginning in the face and jaws and extending rapidly to the right foreleg and right hindleg. These were followed by generalized spasms in which the animal fell to the left, then turned on its back while having sharp clonic spasms of the muscles of the jaw and neck, and pulling its head sharply to the right and voiding urine. Following this, it lay limp, insensible to stimuli for fifteen seconds, when spasms recurred. It was observed continuously for one hour, during which time it had severe recurrences of tonic and clonic spasms resembling "status epilepticus."

June 7: 4 A.M. and 7 A.M.—Attacks of spasms recurred at short intervals but were less severe. Mild attacks of spasms continued through June 12, and the animal was found dead June 13.

The characteristic cloudy swelling and ischemia of brain was found at necropsy. The meninges were wet and shiny. There was no mark at the point of injection. The viscera were normal. Cultures of the brain were negative on blood agar but yielded the streptococcus in dextrose-brain broth. The blood proved sterile.

Protocol 8—A white mouse weighing 25 gm. was inoculated intracerebrally June 11 and 12, 1946, at 9 A.M. with 0.03 ml. of a 20:1 suspension in sodium chloride solution of the heat-killed streptococcus isolated from the brain of a mouse inoculated with the strain isolated from persons having epilepsy in the seventh mouse passage.

June 12: 9:30 P.M.—Severe tremors and slight spasms had developed. 10:30 P.M.—The animal had a generalized convulsion.

June 13: 4 A.M. to 10 A.M.—Th animal was seen to have almost continuous tonic and clonic spasms resembling "status epilepticus," and at 11:45 A.M. the animal died during a severe seizure. Necropsy revealed moderate cloudy swelling of brain, but there were no other lesions. Cultures of brain and blood proved sterile.

Protocol 9—A white mouse weighing 30 gm. was inoculated intravenously June 3, 1946, with 0.5 ml. and intracerebrally with 0.03 ml. of a filtrate of a dextrose-brain broth culture of the streptococcus from the end point of growth of a serial dilution culture from the nasopharynx of a patient suffering from severe epilepsy.

June 4: 4:30 A.M.—Th animal seemed well and was free from spasms. 10 A.M.—As animal was observed, undisturbed, it suddenly developed violent tonic and then clonic generalized convulsions lasting for about fifteen seconds. It then lay quietly, insensible to stimuli for about ten seconds, when it suddenly became rigid with severe tonic and then clonic spasms. It again lay quietly on its side, breathing violently for a few seconds, insensible to stimuli when a third similar seizure resembling

"status epilepticus" occurred, in which it died. Moderate congestion of the cerebral cortex was found at necropsy. Cultures of brain and blood proved sterile.

Protocol 10—A white mouse weighing 25 gm. was inoculated intranasally on June 18, 19, and 20, 1946, with 0.1 ml. of a 20:1 suspension of the live streptococcus after six animal passages and originally isolated from the nasopharynx of persons suffering from severe epilepsy. In addition, it was inoculated intracerebrally with 0.03 ml. of sterile sodium chloride solution immediately after the first intranasal inoculation.

June 21: Until this time the animal remained seemingly well. 7:15 A.M.—At this time of the day after the third intranasal inoculation, it was seen to have violent generalized tonic and then clonic spasms, in which the animal was thrown violently about as the head was drawn sharply backward and as extremities were outstretched in tonic spasms. This attack was followed shortly by rapidly recurring clonic spasms, grinding of teeth, and voiding of urine. The animal then lay limp, insensible to stimuli for ten seconds, when slight and later violent spasms recurred. Seizures resembling the one described then recurred in rapid succession for two hours, after which spasms became continuous resembling "status epilepticus" in which the animal died four hours later. There was slight congestion and cloudy swelling of the brain; the lungs and other viscera were normal. Cultures on blood agar from brain and blood proved sterile, and cultures from the brain in dextrose-brain broth yielded a pure culture of streptococcus.

COMMENTS AND SUMMARY

The occurrence of spasms or convulsions and of disorientation in monkeys in low incidence following inoculation of natural and experimental poliomyelitic and of encephalitic virus and neurotropic streptococci isolated from these and other sources, and the production of respective, more or less characteristic, symptoms in a high incidence in monkeys, rabbits and mice with alpha streptococci isolated from nasopharynx or blood of persons suffering from epilepsy and schizophrenia is reported.

The seizures, especially in mice following inoculation of the streptococci isolated from nasopharynx or blood of persons suffering from epilepsy, resembled those that occur spontaneously in patients in the periodicity, in the suddenness of onset of recurring seizures, in the rapid increment of spasms during attacks ending often in generalized convulsions, a falling to the side, voiding of urine, loss of consciousness, and rapidity of apparent recovery from recurring attacks.

Spasms and convulsions occurred in mice in about equally high incidence on intracerebral, and in comparable low incidence on intranasal, inoculation of the streptococci isolated from nasopharynx of persons suffering from different types of epilepsy — grand mal, petit mal, and psychomotor seizures with mental deterioration without convulsions—but due to prior seizures typical of epilepsy, and from epileptics in whom seizures were controlled by the administration of anticonvulsant drugs—phenobarbital or dilantin or both.

The symptoms in animals that followed the inoculation of the streptococcus isolated from persons suffering from idiopathic epilepsy and dementia paralytica were obviously more convincing proof of etiologic relationship than those following inoculation of the streptococci isolated from persons suffering from schizophrenia.

The occurrence of spasms and convulsions in the baby mouse shown to be due to the streptococcus whose mother had been inoculated intranasally early during gestation with the streptococcus from an epileptic should not be interpreted as indicating either an in utero transmission of the streptococcus or an inherited predisposition, but should rather be interpreted as due to chance nasal-to-nasal infection after birth.

The data obtained indicate that persons suffering from idiopathic epilepsy and schizophrenia harbor in their nasopharynx and sometimes in their blood respective specific types of alpha streptococci, and that the symptoms characteristic of these diseases may be due in large part to the absorption of respective specific neurotoxic substances produced by the streptococci in nasopharynx or other atria of infection in addition to the localization and infection in the brain by the respective streptococci.

The negative cultures from the brain of animals that died from convulsive seizures resembling "status epilepticus" long after intracerebral or intranasal inoculation of the streptococci, the sharp drop of streptococcal antigen in skin or blood with concomitant sharp rise of specific antibody and transient disappearance of symptoms in patients following grand mal seizures, and the subsequent day-by-day increase of antigen (to be reported elsewhere) support the view generally held that the seizures and other symptoms in epilepsy are of toxic origin and that the streptococcus which we have isolated may be a common source of the specific neurotoxin.

A week or ten days after intracerebral, and especially after intranasal, inoculation of material containing the respective specific types of streptococci and negative cultures from the brain of persons for which the "neurotoxin" and the respective specific types of streptococci have predilection or elective affinity, animals may become sensitized so that extremely small amounts of the "neurotoxin" —too small to be detected in serum or blood—and small numbers of streptococci suffice to produce the respective characteristic symptoms.

The common occurrence of the respective specific types of streptococci in the blood of epileptics, especially shortly before or during grand mal seizures, and their occasional presence in the blood of persons suffering from severe schizophrenia are in accord with this idea.

The importance of heredity in relation to the presence of specific types of streptococci in epilepsy and schizophrenia, their relation to the electroencephalogram,[43] the nature of the respective streptococcal "neurotoxins," and active and passive immunization with specific streptococcal vaccines and thermal antibodies[47, 48] are under study.

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