FURTHER STUDIES ON MUSCULAR SPASMS DURING GENERAL ANESTHESIA. EXPERIMENTAL RESULTS WITH NEUROTROPIC STREPTOCOCCI FROM NASOPHARYNGES OF PATIENTS

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IN a previous study it was found that clinical or subclinical infection by a highly specific neurotropic type of alpha streptococcus may be causative of convulsions which sometimes occur during general anesthesia (1). A review of the literature was reported at that time, and since by one of us (Lundy) (2). It is the purpose of this paper to report the results obtained in a further study of the problem.

MATERIAL AND METHODS

The anesthetic agent used on patients, the nature of the spasms and the conditions under which they developed during general anesthesia, and protocols of cases studied experimentally have been given in the previous report and hence are omitted here. In twenty-eight of the forty cases studied in this series, in which muscular spasms occurred during general anesthesia, the patients were observed at the Mayo Clinic and in twelve cases they were observed elsewhere. We are indebted to Dr. R. M. Tovell, Hartford, Connecticut, for sending swabbings from five patients who had convulsions during ether anesthesia, to Dr. R. H. Barrett, Hanover, New Hampshire, for swabbings from three cases, to Dr. H. O. Brown, Chicago, Illinois, for swabbings from two cases, and to Dr. Frank Murphy, Detroit, Michigan, and Dr. H. F. Bishop, Valhalla, New York, for sending swabbings from one case each.

Nasal and nasopharyngeal swabbings of patients of the clinic were made by a member of the Section on Anesthesia and swabbings of the other patients were made by the respective physicians mentioned. The swabbings made in Rochester were cultured immediately whereas those from elsewhere were sent by air mail to the laboratory for study. In some of the cases, second or third nasopharyngeal swabbings and

samples of blood were obtained one or more days later and cutaneous tests were made with the euglobulin fraction of different antistreptococcic serums.

In twenty-eight of the cases the spasms occurred during the spring and summer months. In six cases they occurred during December, January and February, in thirteen cases they occurred during March, April and May, in fifteen cases they occurred during June, July and August, and in six cases they occurred during September, October and November. Aside from the concurrent high incidence of persistent epidemic and postoperative hiccup in cases in which spasm-producing streptococci were isolated, suggesting that a similar type of streptococcus was generally prevalent, there was no apparent reason for the development of the spasms. The isolation of spasm-producing streptococci in a case in which muscular spasms developed during general anesthesia for appendectomy soon after an attack of epidemic hiccup led to the study of this problem from the standpoint of specificity of streptococci (1).

Muscular spasms occurred unexpectedly in the present group of patients during general anesthesia produced with ether and other general anesthetic agents, irrespective of type, duration or magnitude of the surgical intervention for which general anesthesia was administered, and irrespective of age and sex of the patients and presence or absence of an infection associated with fever. Muscular spasms developed during minor and major operations such as tonsillectomy, nasal operations, herniotomy, appendectomy, incision for psoas abscess, correction of webbed fingers, splenectomy, gastric resection for carcinoma and ulcer of the stomach, gastro-enterostomy for ulcer, cholecystectomy, hysterectomy, and excision of osteosarcoma of the femur and chondroma of the scapula. Intravenous injection of only 3 cc. of a 2.5 per cent solution of pentothal sodium usually sufficed to control the spasms permanently.

Cutaneous tests (3) on patients were made by injecting into the skin, as superficially as possible, 0.03 cc. of a 10 per cent solution of the euglobulin fraction of the serum of horses immunized with streptococci isolated in studies of encephalitis, influenza, poliomyelitis and arthritis, respectively. An immediate (five to ten minutes) erythematous reaction 2 sq. cm. or more in diameter was considered to indicate the presence in skin or blood of streptococcal antigen due to infection in nasopharynx or elsewhere by streptococci immunologically related to the streptococcus with which the antibody injected was prepared.

Interphase precipitation tests (3) were made by layering the cleared washings of nasopharyngeal swabbings or the serum of patients on top of the different undiluted antistreptococcic serums in small precipitation tubes. Readings were made after the setups had been incubated at 35 C. for one and a half hours and kept at 10 C. overnight. Clouding at the interphase was considered as indicating a positive

reaction due to the presence of specific streptococcal antigen in nasopharynx or blood serum.

Agglutination tests (3) were made by mixing 0.2 cc. of suspensions containing 4,000,000,000 streptococci per cubic centimeter in saline solution containing 0.2 per cent phenol, and 0.2 cc. of tenfold dilutions in saline solution containing 0.2 per cent phenol, of the respective antistreptococcic serums (1:10 to 1:10,000). The setups were incubated at 50 C. for eighteen to twenty-four hours, at the end of which period readings were made.

Cultures were made by inoculating dextrose-brain broth and dextrose-brain agar in tall tubes and blood-agar plates with washings in 2 cc. of 0.2 per cent gelatin Locke solution of nasal or nasopharyngeal swabbings of patients. Nasal swabbings made through the nares were nearly always contaminated with staphylococci and hence were not inoculated routinely. All cultures were incubated at 35 C.

At first 1:200 to 1:2,000 dilutions of the primary dextrose-brain broth cultures were inoculated into animals. Later, 1:10,000 dilutions in dextrose-brain broth of the end point of growth of streptococci in serial dilution (4) cultures made alternately in dextrose-brain broth and dextrose-brain agar at steps of 10^{-4} at each transfer, were inoculated.

The dextrose-brain broth consisted of 0.2 per cent ordinary dextrose broth adjusted to pH 7.2, and the soft dextrose-brain agar also used likewise consisted of dextrose broth containing 0.2 per cent agar, to both of which approximately one part of freshly obtained calf brain to six or seven parts of broth in tall (10 cm.) columns was added before autoclaving.

Rabbits weighing from 1.5 to 2 kilograms were inoculated intracerebrally into the right frontal lobe with 0.1 cc. of the gelatin Locke solution washing of the nasopharyngeal swabbing, and rabbits and guinea-pigs were inoculated intracerebrally with 0.1 cc. of the 1:200 to 1:10,000 dilutions of primary, sometimes mixed dextrose-brain broth cultures, or with the pure culture at the end point of growth in serial dilution cultures. The monkey was inoculated intracerebrally with 1.5 cc. of a 1:200 dilution of a culture of the living streptococci and with 2 cc. of a suspension of the dead streptococci. Rabbits were inoculated intravenously with 0.5 cc. per kilogram of body weight of the dextrose-brain broth cultures and with 1 cc. into the upper cancellous part of the tibia. The intratibial inoculation was done by removing the hair, sterilizing the skin, puncturing the tibia wth a sterilized phonograph needle fitted into a brass handle, and inserting a 23 gage needle 6 mm. in length with blunt end projecting through a rubber disk. Injection was made under pressure by pressing the rubber disk firmly against the punctured area to prevent regurgitation of the culture while it was being injected.

Mice were inoculated intracerebrally with 0.03 cc. of highly diluted

dextrose-brain broth cultures of the streptococci, intraperitoneally with 1.2 cc. and intranasally with 0.05 cc. of the undiluted culture.

Intracerebral and intratibial inoculations were carried out while the animals were under ether anesthesia.

Animals were observed two or more times daily. In the case of animals that died from effects of inocula or that succumbed suddenly during anesthesia, cultures were made routinely in dextrose-brain broth from pipettings of the brain and of blood from the heart.

Mallinckrodt's ether was the anesthetic agent chiefly used to produce general anesthesia in animals but in a few instances a mixture of nitrous oxide and oxygen was used. A rectangular glass jar (15 by 16 by 5½ inches [38 by 41 by 14 cm.]) with several layers of cloth in the bottom, fitted with a loosely fitting lid having a hole 2 cm. in diameter in the center above a screened compartment filled with absorbent cotton or cloth, was used as an etherizing chamber. Ether from freshly opened cans or from tightly stoppered cans was poured into the jar and onto the cotton under the lid immediately before the animals were placed in the jar. After induction of anesthesia the animals were removed from the jar and anesthesia usually was continued by means of a modification of the open cone method. This consisted of causing animals to breathe ether vapor through ether-soaked cotton placed at the bottom of an empty ether can which contained many perforations. The can was held loosely over the nose of the animal to maintain the desired degree of anesthesia. Thus, undue concentration of carbon dioxide from rebreathed air (5) was prevented. However, in some instances, anesthesia was produced by placing ether-soaked cotton in paper bags, which were held tightly around the neck of animals with rubber bands to increase the concentration of carbon dioxide, but with this method of etherization there was no noticeable increase of incidence or degree of spasms. At first, rectal temperatures of rabbits were taken at the time ether was administered but since there was no correlation between spasms and fever and because the temperature was so variable, this procedure was discontinued.

RESULTS OF CULTURES

Blood-agar plates made directly from washings of nasopharyngeal swabbings and from dextrose-brain broth cultures almost always revealed a great preponderance of green-producing or alpha streptococci. In serial dilution cultures, streptococci having specific properties grew in much higher dilution than did other bacteria and saprophytic streptococci or streptococcic variants of the specific strains. Hemolytic streptococci from nasopharynges sometimes grew in small numbers on blood-agar plates but they never were obtained in the serial dilution cultures made alternately in dextrose-brain broth and dextrose-brain agar.

RESULTS AMONG ANIMALS

A fairly characteristic group of symptoms and findings developed in from twelve to seventy-two hours among rabbits, guinea-pigs and a monkey, following intravenous (protocol 1), intracerebral (protocols 2 to 5), and intratibial (protocol 6) inoculation of the streptococci from the patients who had ether convulsions. Tremors, twitchings and clonic spasms of muscles were the most striking symptoms. These

TABLE 1

RESULTS, BEFORE AND AFTER ETHER ANESTHESIA, AMONG RABBITS INOCULATED WITH A STREPTOCOCCUS ISOLATED FROM THE NASOPHARYNX OF A PATIENT IN WHOM CONVULSIONS HAD DEVELOPED DURING ETHER ANESTHESIA

Groups and methods of inoculation		Rabbits					
		Inoculated	That had tremors or spasms before ether was given	Given ether anesthesia 1 to 4 days after inoculation			
				Number anesthetized	Anesthesias	Incidence of spasms or convulsions	
						Number	Per cent of anesthesias
Rabbits inoculated with dextrose-brain broth culture of streptococci from nasopharynx of a patient in whom convulsions had developed during ether anesthesia	Intracerebral	14	12	13	43	38	88
	Intravenous	4	2	4	9	9	100
	Intratibial	6	3	6	16	14	88
Uninoculated rabbits or rabbits that had recovered from previous inoculations of streptococci from other sources		15	0	15	26	1	4

symptoms were usually milder and less generalized and progressed less often than did similar symptoms which occurred after inoculation of streptococci isolated in studies of persistent epidemic and postoperative hiccup and myoclonic encephalitis.

When the inoculated animals, especially rabbits, were anesthetized with ether from one to four days after inoculation of the streptococci a remarkably constant and characteristic train of symptoms developed, both in those cases in which tremors or spasms had developed before ether anesthesia (protocols 2, 3, 4 and 6) and in those in which tremors or spasms had not developed before ether anesthesia (protocols 7 and 8). During induction of anesthesia, far greater evidence of excitation and generalized spasms occurred than in uninoculated or recovered

rabbits similarly treated. After a period of breath-holding, respirations became rapid and, as anesthesia became complete, clonic spasms of muscles developed, beginning usually in the lips and nose and extending rapidly backward to muscles of eyelids, ears, neck, shoulders, fore extremities, trunk, hind extremities and tail. If administration

TABLE 2

RESULTS AMONG ANIMALS FOLLOWING INOCULATION OF STREPTOCOCCI ISOLATED FROM PERSONS IN WHOM CONVULSIONS HAD DEVELOPED DURING GENERAL ANESTHESIA, AND WITH STREPTOCOCCI FROM OTHER SOURCES

Streptococci isolated in studies of			Cases or strains	Results in animals not anesthetized								
				Inoculated	Died, per cent	Symptoms, per cent				Cultured		
						Tremors	Spasms		Flaccid paralysis	Number	Percentage incidence of isolation of streptococci from	
							Diaphragm	Other muscles			Brain	Blood
"Ether convulsions"	Nasopharynx	Washings	17	17	35	[100]	0	[94]	0	10	70	0
		Dextrose-brain broth cultures	40	166	65	[64]	5	[70]	7	107	59	15
		Suspensions of heat-killed streptococci	8	8	0	[100]	0	[88]	0	7	0	0
		Total	40	191	59	[69]	4	[73]	6	124	[56]	13
Encephalitis, multiple sclerosis, persistent epidemic and post-operative hiccup (nasopharynges of patients)			55	76	41	[68]	3	[63]	13	44	[70]	7
Poliomyelitis (nasopharynges of patients and spinal cords of monkeys)			45	61	48	48	0	23	[51]	35	[74]	11
Miscellaneous diseases other than of the nervous system (atria of infection of patients)			115*	114	33	32	0	14	9	39	44	5

* Pools of two or more strains were inoculated in some instances.

of the anesthetic agent was discontinued promptly as these spasms occurred, the more generalized spasms usually disappeared and recovery ensued. However, if administration of the anesthetic agent was continued, even for a short time after spasms began, they often became violent, respiration ceased suddenly and death occurred from

cardiac failure. If action of the heart continued, restoration of respiration was accomplished by drawing the tongue forward and doing artificial respiration (protocol 2). During about a fourth of the anesthesias the muscular spasms began in the hind extremities and extended rapidly forward. After anesthesia had been repeated, descending and ascending spasms sometimes occurred in the same animal.

TABLE 3

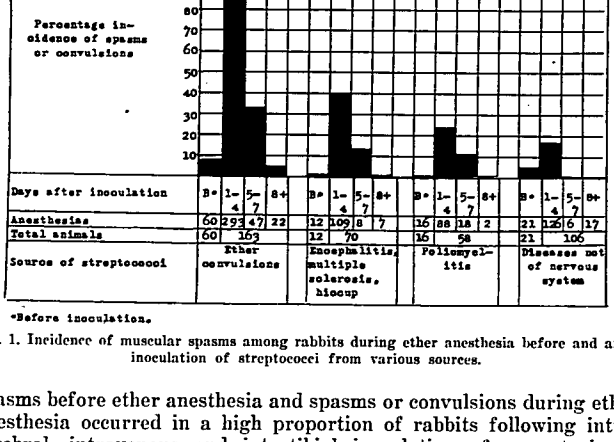
RESULTS AMONG RABBITS AFTER INOCULATION OF A STREPTOCOCCUS ISOLATED FROM THE NASOPHARYNX OF A PATIENT IN WHOM CONVULSIONS HAD DEVELOPED DURING GENERAL ANESTHESIA WHILE OPERATION FOR INTRACTABLE ULCER OF THE DUODENUM WAS BEING PERFORMED

Source of streptococci		Animals			Ether anesthesias 1-2 days after inoculation		
			Per cent showing			Anesthesias	
		Inoculated	Tremors or spasms before ether anesthesia	Hemorrhage, ulcer, or both, of stomach or duodenum	Animals anesthetized	Number	Percentage incidence of spasms or convulsions
Nasopharynx of patient, three daily swabbings		14	79	71	12	16	88
Brains of inoculated animals		6	83	33	6	8	75
Lesions in mucous membrane of stomachs of inoculated animals		6	0	83	6	8	25
Heat-killed streptococci and filtrate of dextrose-brain broth culture of streptococci from nasopharynx		5	100	60	5	8	100
Controls	Heat-killed streptococci and filtrate of dextrose-brain broth culture of poliomyelitis virus	5	80	0	5	8	13
	Nasopharynges of well persons; poliomyelitis and equine encephalomyelitis virus	14	64	7	14	18	17

On recovery from anesthesia the tremors or spasms recurred if they had been present prior to anesthesia. If they had been absent before anesthesia, they usually were absent after it. However, in some instances, anesthesia seemingly caused them to appear and to continue. Among rabbits having severe spasms after inoculation of the streptococcus, intravenous injection of pentothal sodium, equivalent in amount per kilogram of body weight to that which effectively controls spasms among patients, caused the spasms to disappear promptly, although they soon returned. On the other hand, among rabbits having slight

spasms identical injections of pentothal sodium often caused the spasms to disappear permanently. Ether administered immediately after injection of pentothal sodium failed to produce spasms.

The results obtained before and after administration of ether to animals inoculated with streptococci isolated in studies of cases of ether convulsions and from other sources are summarized in tables 1, 2 and 3 and in figure 1. As shown in table 1, tremors or muscular



*Before inoculation.

FIG. 1. Incidence of muscular spasms among rabbits during ether anesthesia before and after inoculation of streptococci from various sources.

spasms before ether anesthesia and spasms or convulsions during ether anesthesia occurred in a high proportion of rabbits following intracerebral, intravenous and intratibial inoculation of one strain of streptococci isolated in a study of a case of ether convulsions. This was not true of uninoculated rabbits or of rabbits that had recovered from previous inoculations of streptococci from other sources.

The mortality rate, symptoms and incidence of isolation of streptococci from brain and blood of animals after inoculation of the streptococci isolated in studies of forty cases of ether convulsions and from other sources are summarized in table 2. It will be seen (1) that the mortality rate was highest in the ether convulsion group (59 per cent); (2) that the incidence of tremors (69 per cent) and spasms of muscles (73 per cent) was about the same in this group as in the encephalitis group (68 and 63 per cent), less in the poliomyelitis group (48 and 23 per cent) and least in the miscellaneous group (32 and 14 per cent) and (3) that the incidence of flaccid paralysis was much higher in the poliomyelitis group (51 per cent) than in the other groups (6, 13 and 9 per cent, respectively). The incidence of isolation of

streptococci from the brain was highest in the poliomyelitis group (74 per cent), second highest in the encephalitis group (70 per cent), next highest in the ether convulsion group (56 per cent) and least in the miscellaneous group (44 per cent).

The incidence of spasms or convulsions among animals during ether anesthesia before inoculation and one to four, five to seven and eight or more days after inoculation of streptococci isolated, respectively, in studies of ether convulsions, encephalitis, multiple sclerosis, hiccup, poliomyelitis and miscellaneous diseases other than those of the nervous system is shown in figure 1. The incidence of spasms or convulsions was by far the highest after inoculation of streptococci isolated in studies of ether convulsions, next highest in the encephalitis group, lower in the poliomyelitis group and least in the group that had received streptococci similarly isolated in studies of diseases other than those of the nervous system. The graph indicating the incidence of muscular spasms during ether anesthesia after inoculation of streptococci from patients in whom spasms or convulsions had occurred includes seven animals that received direct intracerebral inoculation of gelatin Locke solution washings of nasopharyngeal swabbings and five animals that had received the heat-killed streptococci. In the former, the incidence of spasms during thirty-four ether anesthesias was 71 per cent and in the latter, 87 per cent during fifteen anesthesias.

The incidence of spasms during ether anesthesia among thirty-nine additional normal rabbits that were not inoculated subsequently with streptococci was 5 per cent.

Nine rabbits were inoculated intravenously with three different strains isolated in studies of cases of spasms during general anesthesia. One of these rabbits died in general convulsions before ether was administered. Eight rabbits survived the effects of the injection and tremors and clonic spasms of masseters and muscles of neck developed in six. Ether anesthesia was administered one or more times (fourteen in all) to the eight rabbits one to two days after inoculation. Moderate to severe spasms developed in each of the eight rabbits.

Streptococci from the nasopharynges of ten patients who had ether convulsions were inoculated into altogether fifty-two mice. Thirteen mice were inoculated intracerebrally. Twelve of them succumbed and tremors or spasms were observed in six. Eighteen mice were inoculated intraperitoneally; seven of them succumbed. Twenty-one mice were inoculated intranasally and two succumbed. Lesions of lungs were not observed in any of these animals.

In accord with results reported previously, dual effects were noted among animals inoculated intracerebrally with the streptococci isolated from patients suffering from acute appendicitis or intractable ulcer of the stomach or duodenum. Thus, in addition to producing tremors or spasms before ether was given and spasms or convulsions during ether anesthesia, the streptococci localized and produced lesions elec-

tively in the appendix (protocols 1 and 2), stomach or duodenum of animals (protocols 4 and 5).

As shown in table 3, inoculation of streptococci from the nasopharynx of a patient in whom muscular spasms had developed during general anesthesia while operation was being performed for intractable ulcer of the duodenum produced a high incidence of tremors or spasms (79 per cent) before administration of ether and of spasms or convulsions (88 per cent) during ether anesthesia and a high incidence of lesions of stomach or duodenum (71 per cent) (figs. 2 and 3).

FIG. 2. Ulcer of the stomach and congestion of the duodenum of a rabbit inoculated intracerebrally with a culture of streptococcus obtained from the nasopharynx of a patient in whom ether convulsions had developed during general anesthesia while operation for ulcer was being performed.

Inoculation of the streptococci isolated from the brains of animals that had succumbed produced, in turn, a high incidence of tremors or spasms (83 per cent) before, and spasms or convulsions (75 per cent) during, ether anesthesia and a low incidence of lesions of the stomach or duodenum (33 per cent). On the other hand, inoculation of the streptococci isolated from the lesions in the stomach produced only a high incidence of lesions of the stomach or duodenum (83 per cent)

(protocol 9). The heat-killed streptococci and filtrates of dextrose-brain broth cultures of the streptococci from the nasopharynx of this patient produced a high incidence of tremors or spasms (100 per cent) before ether was given, a high incidence of spasms during anesthesia (100 per cent) and a high incidence of lesions of the stomach or duodenum (60 per cent). Among control rabbits there was a high incidence of tremors or spasms (80 and 64 per cent, respectively) after inoculation of heat-killed streptococci and filtrates of dextrose-brain

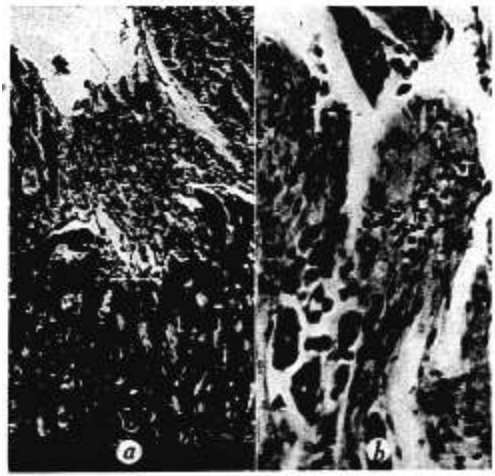


FIG. 3a. Section of the ulcer shown in figure 2; *b*, leukocytic infiltration in the base of the sloughing tissue shown in *a* (*a*, hematoxylin and eosin stain × 70, and *b*, hematoxylin and eosin stain × 245).

broth cultures and of living streptococci from nasopharynges of well persons and from poliomyelitis and equine encephalomyelitis virus, whereas the incidence of spasms or convulsions during ether anesthesia (13 and 17 per cent, respectively) and of lesions of stomach or duodenum (0 and 7 per cent) was low.

The gross and microscopic lesions and localization of the streptococcus that occurred in the stomach of animals after inoculation of the streptococci isolated from the patient in whom spasms had developed during general ether anesthesia while operation for intractable ulcer was being performed are well illustrated in figures 2, 3 and 4. The microscopic lesions and localization of the streptococcus in the brain of animals after inoculation of the streptococcus isolated from the naso-

pharynx of patients in whom spasms had developed during ether or other general anesthesia are typified in figures 5 and 6. Lesions of the brain were highly characteristic, consisting of a variable degree (depending on the duration of the experiment) of non-suppurative meningitis associated with parenchymatous and perivascular leukocytic and round-cell infiltration, with or without necrosis, in the gray matter of the cerebral cortex or subcortex and sometimes of the ependyma. Lesions such as those shown in figure 5 *a* and *b* were especially common after inoculation of these streptococci but almost never have been observed after inoculation of streptococci from other sources.

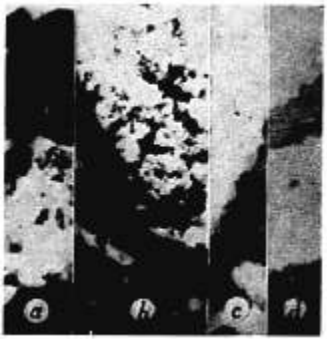


FIG. 4. Streptococci and diplococci in the tissues at the base of ulcers of the stomach that developed among rabbits and guinea-pigs after inoculation of the strain referred to in figure 2: *a*, in a rabbit, *b*, in a guinea-pig, *c*, in a rabbit inoculated with the streptococcus isolated from an emulsion of the hemorrhagic ulcer shown in figure 2, and *d*, in a rabbit inoculated with the streptococcus isolated from an emulsion of the ulcer that had developed in the rabbit referred to in *c* (Gram stain × 1,000).

ILLUSTRATIVE EXPERIMENTS AND PROTOCOLS

On July 13, 1939, three rabbits were inoculated, one intravenously with 10 cc. and two intracerebrally with 0.1 cc., of a 1:200 dilution of a dextrose-brain broth culture of streptococci from the nasopharynx of a patient in whom muscular spasms had developed during anesthesia while operation for acute appendicitis was being performed. On July 18 a fourth rabbit was inoculated intracerebrally with this strain in the fourth subculture after one animal passage. Tremors and muscular spasms developed in all of the four animals and muscular spasms developed in three during ether anesthesia. Lesions of the appendix developed in three of the rabbits. The following protocols will serve to illustrate the course of events in the animals inoculated.

Protocol 1.—A white rabbit was inoculated intravenously on July 13, 1939, at 11 a.m. At 4 p.m. the animal appeared well. At 9 p.m. spasms of muscles

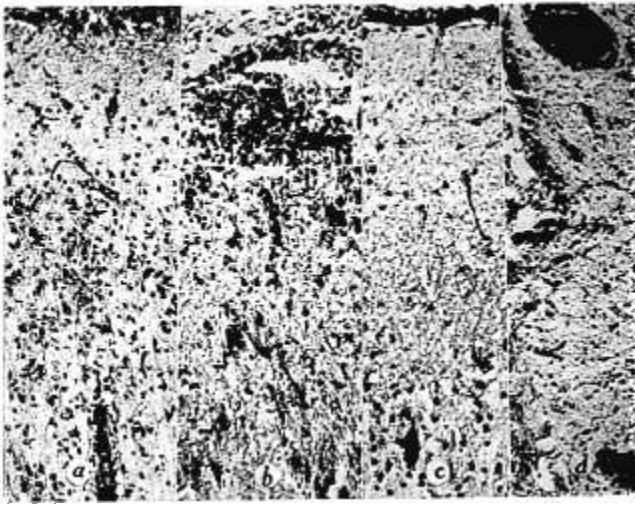


FIG. 5. Lesions of the brain of four rabbits (*a*, *b*, *c*, and *d*,) in which characteristic spasms had developed during ether anesthesia, two, three, two and eight days, respectively, after intracerebral inoculation of four strains of streptococci isolated from the nasopharynx of four patient in whom convulsions had developed during general ether anesthesia (hematoxylin and eosin stain × 170).

FIG. 6. Diplococci and streptococci in the lesions of the brains of animals in which spasms had developed during ether anesthesia following inoculation of streptococci, *a* and *b*, in section *a* of figure 5, *c* in section *b* of figure 5, and *d* in section *c* of figure 5 (Gram stain × 1,000).

of the face and ears were noted. On July 14 the animal was found dead in a position as if it had died from convulsions. Necropsy revealed extreme congestion of the brain, marked degeneration of the liver, cloudy swelling and severe congestion and hemorrhages in the appendix, hemorrhagic and edematous lymph nodes at the base of the appendix and no lesions of stomach, kidneys or bladder. Cultures from the blood and brain yielded a pure growth of green-producing streptococci.

Protocol 2.—A white rabbit was inoculated intracerebrally on July 13, 1939, at 11 a.m. At 4 and 8 p.m. the animal appeared quite well. On July 14 at 8 a.m., ataxia and sharp, generalized clonic spasms, especially on prodding, were noted. At 9:30 a.m. the animal sat quietly in its cage with increased respiration. When it was prodded, ataxia, weakness of muscles of the neck, occasional sharp spasms of muscles of the ears, slight spasms of muscles of the extremities and great hyperirritability were noted.

At 9:40 a.m. the animal was given ether. No spasms developed during induction of anesthesia. After complete anesthesia the animal lay quietly on its side, breathing normally for a time. Suddenly the respirations became increased as spasms of muscles of the entire body developed, associated with cessation of respiration. Palpation revealed that the heart was still beating. When the tongue was drawn forward and artificial respiration was administered, the animal began to breathe again. While it was still lying on its side sharp spasms of the muscles of the face and eyelids, and occasionally of the ears, developed. After about ten minutes the condition of the animal was similar to that before anesthesia. At 4:40 p.m. there was no change in its condition. Ether was administered very carefully. There were no spasms until the animal was under deep anesthesia. Then spasms began in the muscles of the face and spread backward, involving muscles of the ears, neck, eyelids, hind extremities and tail in rapid succession. The spasms continued for some time. The animal was allowed to recover from the anesthetic agent and again etherized. No spasms developed during induction of anesthesia but severe spasms occurred during deep anesthesia and respirations ceased suddenly. When artificial respiration was administered, generalized spasms of muscles of ears, face and extremities developed. At 5:30 p.m. the animal was anesthetized for the fourth time. Severe generalized spasms again developed during deep anesthesia and respirations ceased. Palpation revealed that the heart had stopped beating and artificial respiration proved of no avail.

Necropsy revealed severe congestion of the appendix, marginal hemorrhagic edema of lungs and bronchopneumonia, congestion of mucous membrane of the trachea and moderate congestion of the brain. A pure culture of streptococci was isolated from the brain. Cultures from the blood proved negative.

Protocol 3.—A white rabbit was inoculated intracerebrally at 9:30 a.m. on February 1, 1940, with 0.1 cc. of a 1:2,000 dilution of a dextrose-brain broth culture of the nasopharyngeal swabbing of a patient in whom convulsions had developed during general anesthesia while operation for carcinoma of the stomach was being performed. At 4:30 p.m. the animal appeared well but when its head was held under tension severe tremors and spasms of masseters were noted.

At 4:45 p.m. the animal was placed in the ether chamber and during induction of anesthesia great excitation and generalized spasms developed. The respirations became violent and this change was associated with extreme dilatation

of pupils and congestion of the conjunctiva and of the vessels of the sclera. Soon after recovery from anesthesia the animal was placed again in the ether chamber; during induction of anesthesia, spasms of the muscles of the jaw developed and during deep anesthesia generalized spasms occurred. As the animal recovered from anesthesia the spasms disappeared. At 7:30 p.m. continuous spasms and tremors were present. During ether anesthesia spasms of the eyelids and ears and horizontal nystagmus developed. At 9:30 p.m. tremors and spasms were still present. When the animal was placed in the etherizing chamber for the fourth time violent, generalized, clonic spasms developed during induction of anesthesia. These disappeared during deep anesthesia. During recovery from anesthesia spasms of the masseters and of the muscles of the eyelids and tail developed and respirations again became violently increased.

On February 2 at 9 a.m., the animal was ataxic, occasional spasms of muscles of the ears occurred when it was sitting quietly and tremors and spasms of masseters were still present. Respirations were normal. On prodding, marked ataxia and spasms of muscles of eyelids, neck and face developed. At 2:30 p.m. the animal sat quietly in its cage without spasms. As the animal was carried to an adjacent room no spasms developed. Almost immediately after it had been placed in the etherizing chamber violent, almost fatal, generalized spasms developed. As the animal was removed from the etherizing chamber the respirations became labored and clonic spasms of muscles of neck, shoulders and back occurred. The spasms disappeared after recovery from anesthesia. On February 3 the animal appeared quite well although somewhat tremulous. During induction of ether anesthesia severe generalized spasms developed. These continued for some time but as the animal recovered from anesthesia the spasms disappeared. On February 5 the animal was found dead. Necropsy revealed severe congestion of the brain and of the mucous membrane of the trachea and hemorrhagic edema and emphysema of lungs. Cultures from brain and blood yielded streptococci.

Protocol 4.—A white rabbit was inoculated intracerebrally on March 5, 1941, with 0.1 cc. of gelatin Locke solution suspension of the nasopharyngeal swabbing from a patient, referred to in table 3, on whom an operation for intractable ulcer had been performed. The rabbit had not had any spasms during ether anesthesia before inoculation. On March 6 at 9:30 a. m., the animal was tremulous and when it was prodded, generalized, clonic spasms developed, lasting for a short time. Respirations were moderately increased. During induction of ether anesthesia there were no spasms but after prolonged deep anesthesia sharp clonic spasms of muscles of abdomen, back and hind extremities occurred. These gradually increased and extended to the fore extremities. Soon after recovery a second ether anesthesia was administered. During deep anesthesia typical spasms began in muscles of the face and extended rapidly to muscles of the ears, fore extremities, back and tail. The animal died suddenly in convulsions during the third ether anesthesia and was examined at once.

Many punctate hemorrhages were found in the pyloric end of the stomach but none in the cardiac end. The lungs were normal and the brain was moderately congested. Cultures of an emulsion of the washed hemorrhagic mucous membrane from the pyloric end of the stomach yielded a pure growth of streptococci in one tube of dextrose-brain broth and the streptococcus in mixture with staphylococci and Escherichia coli in another tube of dextrose-

brain broth. Cultures from an emulsion of the washed mucous membrane of the cardia yielded pure streptococci in one tube of dextrose-brain broth. Streptococci were not obtained in any of six tubes of dextrose-brain broth inoculated with an emulsion of pieces of washed mucous membrane from the pyloric end of the stomach in which no lesions were found. Cultures from pipettings from the brain yielded streptococci.

Protocol 5.—When a normal Macacus rhesus monkey of medium size was etherized on March 6, 1941, no spasms occurred. After recovery from anesthesia it was inoculated intracerebrally with 1.5 cc. of a 1:200 dilution in gelatin Locke solution of a single colony of streptococcus isolated from the end point of growth, representing the fourteenth transfer made with the same wire, in a serial dilution culture of the second nasopharyngeal swabbing of the patient who had intractable ulcer and in whom muscular spasms had developed during anesthesia. On March 7 the animal was slightly excitable. During the course of two ether anesthesias no spasms developed, but as the animal recovered from the second anesthesia spasms of the fore extremities, trunk and then hind extremities developed, associated with rapid vertical nystagmus. On March 8 while the animal was held by the neck and back, severe generalized spasms occurred. It was then etherized. No spasms occurred during induction of, or during, deep anesthesia but as the animal recovered from anesthesia tonic spasms of fore and hind extremities again developed.

On March 11 the animal appeared well. Generalized spasms did not occur during induction of anesthesia but tonic spasms of the fore extremities and clonic spasms of the muscles of the ears and eyelids did occur. As the animal recovered from anesthesia tonic spasms of fore and hind extremities, stertorous breathing with spasm of the glottis, and vertical nystagmus developed. When the animal was grasped over the neck while recovering from anesthesia the spasms became worse and flexion of the fingers was so great that it was almost impossible to extend the fingers by force. Vertical nystagmus and stertorous breathing recurred. On March 12 the animal appeared quite well but the temperature was 101.4 F. At 3:15 p.m. 2 cc. of a suspension (seven times the density of a dextrose-brain broth culture) of heat-killed streptococci (in the third subculture) from the third nasopharyngeal swabbing of the patient was injected intracerebrally. At 7:30 p.m. tremors and clonic spasms of extremities were noted and as the animal recovered from deep ether anesthesia general rigidity developed. On March 13 the animal had severe, sharp clonic spasms of the fore extremities when held by the neck. It was given ether anesthesia and died suddenly.

Necropsy revealed one large hemorrhage in the mucous membrane of the fundus of the stomach and four small erosions surrounded by opaque, swollen mucous membrane resembling beginning peptic ulcer. A small hemorrhagic cyst was found at the point of injection in the left frontal lobe. Cultures from the blood proved sterile; those from the brain revealed a pure growth of streptococci.

Protocol 6.—A white rabbit was inoculated intratibially on April 18, 1940, with 1 cc. of a dextrose-brain broth culture of streptococci isolated from the nasopharynx of a patient in whom muscular spasms had developed during anesthesia for an operation for osteosarcoma of the right humerus. The animal had been etherized before inoculation but no spasms had developed. On April 19, the animal appeared well but tremors and spasms and twitchings of masseters

were noted when it was placed under tension. It was given ether anesthesia. Clonic spasms of muscles of the face developed during recovery from deep anesthesia. On April 21, the animal appeared well. During deep ether anesthesia spasms of muscles of the face, eyes and ears developed, associated with nystagmus. On April 23 the animal appeared well and there were no tremors. When it was etherized, tremors, spasms of muscles of face and eyelids and nystagmus developed. During a second anesthesia which was given, these spasms recurred, extending to the fore extremities and back. Recovery ensued. On April 29 the animal appeared well and spasms did not develop during or after recovery from ether anesthesia.

Protocol 7.—A gray and white rabbit was inoculated intracerebrally on May 3, 1942, with 0.1 cc. of a 1:10,000 dilution of a dextrose-brain broth culture of streptococci from the end point of growth (10^{-14}) in a serial dilution culture of a nasopharyngeal swabbing from a patient in whom convulsions had developed during general anesthesia for tonsillectomy. On May 4 at 10 a.m., there were no symptoms other than slight tremors of the masseters. The animal was etherized and during induction of anesthesia violent generalized spasms developed. The animal was allowed to recover. When it was etherized a second time severe spasms again developed during deep anesthesia. These spasms began in the muscles of the face, extended rapidly backward, continued violently for some time and then suddenly disappeared. Shortly after recovery a third anesthesia was given but no spasms developed. On May 5, the animal appeared well but during deep ether anesthesia generalized spasms developed.

On May 6, at 9 a.m., the animal appeared well. Moderately severe spasms developed during deep anesthesia. At 4 p.m. the animal appeared well. A mixture of nitrous oxide and oxygen (90:10) was administered and as the animal fell asleep generalized tremors developed. When the percentage of oxygen was increased the tremors disappeared. The tremors recurred when the proportion of oxygen was decreased and during deep anesthesia generalized spasms developed, including retraction of the head. The wink reflex did not disappear under anesthesia. The animal was allowed to recover and a second nitrous oxide-oxygen (95:5) anesthesia was administered. As before, severe generalized tremors and spasms developed during deep anesthesia and the wink reflex did not disappear. On May 11, the animal appeared well. During two deep ether anesthesias spasms did not occur. On June 3, the animal was found dead. Necropsy revealed an intercurrent infection by Brucella bronchiseptica. There were no gross lesions of viscera.

Protocol 8.—A white Angora rabbit was inoculated intracerebrally on June 5, 1942, with 0.1 cc. of a 1:200 dilution of the first subculture in dextrose-brain broth of a culture from the nasopharynx of a patient in whom spasms had developed during anesthesia for cholecystectomy. On June 6 the animal appeared well. It was given ether. During induction of anesthesia and while the rabbit was under deep anesthesia, severe generalized spasms bordering on convulsions developed. After recovery from anesthesia tremors and mild spasms of the muscles of the abdomen, back, ears, tongue and paws occurred. Ether was administered a second time and generalized spasms developed resembling those that had occurred after the first anesthesia. After complete recovery from anesthesia tremors of the muscles continued for a time. On June 7 the animal was found dead. Examination revealed moderate congestion of the brain but no lesions of viscera. Dextrose-brain broth cultures of pipettings from the brain yielded streptococci.

Protocol 9.—A white rabbit was inoculated intracerebrally on March 17, 1941, with 0.1 cc. of a dextrose-brain broth culture of streptococci isolated from the hemorrhagic mucous membrane of the cardiac end of the stomach of a rabbit in which ulcer had developed after inoculation of streptococci from the nasopharynx of a patient suffering from intractable ulcer, referred to in table 3. The strain had been through two passages in animals. On March 18, only slight twitchings occurred in the muscles of the eyelids during the first, and no spasms occurred during the second, ether anesthesia. The animal died suddenly from cardiac failure and cessation of respiration during the second deep anesthesia. At necropsy severe, diffuse congestion of the vessels of the stomach and hemorrhages in the fundus of the stomach were found. There were no other lesions of viscera. Cultures from the blood were sterile but those from the brain and from the hemorrhagic mucous membrane of the stomach yielded streptococci.

RESULTS OF SEROLOGIC STUDIES

Agglutination tests were made with sixty-eight cultures of streptococci, before or after animal passage, isolated from the nasopharynges of thirty-four of the patients who had convulsions when given ether. Agglutination was maximal and occurred in highest dilution with the encephalitis antistreptococcic serum in sixty-one of the cultures representing the thirty-four cases, with the influenza antistreptococcic serum in five, and with the ulcer and poliomyelitis antistreptococcic serums in one each. Seven of nine cultures from the nasopharynx of the patient suffering from duodenal ulcer in whom muscular spasms developed during general anesthesia were agglutinated maximally by the encephalitis antistreptococcic serum, whereas six of the seven cultures from the lesions in the stomach of inoculated animals were agglutinated maximally by the ulcer antistreptococcic serum.

Precipitation tests were made with cleared washings of forty nasopharyngeal swabbings obtained in twenty-four of the cases and the different antistreptococcic serums prepared in horses in parallel manner. Clouding at the interphase, or a positive reaction, occurred with the encephalitis antistreptococcic serum in thirty instances, with the poliomyelitis antistreptococcic serum in sixteen, with the arthritis antistreptococcic serum in six, with the influenza antistreptococcic serum in twelve, with the ulcer antistreptococcic serum in five and with normal horse serum in two. Precipitation tests were made also with twelve specimens of serum obtained in ten of the cases and the different antistreptococcic serums. Clouding at the interphase occurred with the encephalitis antistreptococcic serum in four, with the poliomyelitis and influenza antistreptococcic serums in two each and with the ulcer antistreptococcic serum in one. A positive reaction was not obtained with the arthritis antistreptococcic serum or with normal horse serum.

Cutaneous tests were made in ten of the cases. Erythematous

reactions covering an area of 3.14 to 12.57 sq. cm., averaging 9.3 sq. cm., occurred in the skin of each of the ten patients at the site of intradermal injection of the encephalitis euglobulin. Reactions ranging from 2.01 to 3.14 sq. cm. occurred in five at the site of injection of the influenza euglobulin. Reactions of 3.14 and 4.91 sq. cm. occurred in the skin of two persons at the site of injection of the arthritis euglobulin and no reaction to this euglobulin occurred in eight. No reactions occurred with normal horse serum diluted 1:10.

Summary

Green-producing streptococci of usual morphologic and cultural characteristics, but which had highly specific properties, were isolated by special methods from the nasopharynges of persons in whom muscular spasms had developed during general anesthesia. Intracerebral, intravenous and intratibial injection of the freshly isolated streptococci and intracerebral inoculation of the dead streptococci and filtrates of actively growing cultures produced a high incidence of tremors and spasms of muscles in rabbits, guinea-pigs, mice and a monkey. Among animals so inoculated, quite regardless of the presence or absence of symptoms, there was a greatly increased tendency, soon after inoculation, for muscular spasms or convulsions to develop during ether and nitrous-oxide-oxygen anesthesia, and as recovery from the effects of the inocula ensued this tendency disappeared.

Tremors and spasms before and during ether anesthesia and selective development of lesions in the stomach, duodenum or appendix of animals after inoculation of living and dead streptococci and filtrates of actively growing cultures of streptococci from nasopharynges of patients in whom muscular spasms developed during anesthesia for operation for ulcer of the stomach or duodenum or appendicitis, were found to be due to a mixture of streptococci having respective specific affinities.

Results of agglutination, precipitation and cutaneous tests with the encephalitis antistreptococcic serum were in accord with the highly specific effects obtained in animals.

Cassels, Becker and Seevers (5) have shown that a high degree of respiratory acidosis produced rapidly by the administration of carbon dioxide may increase cerebral irritability of animals sufficiently to result in convulsions during general anesthesia. Their experiments may afford an explanation of a possible mechanism causing a type of muscular spasms during general anesthesia. In our experiments ether anesthesia was administered in such a manner as to avoid undue concentration of carbon dioxide. Muscular spasms during anesthesia almost always occurred in animals soon after inoculation of the streptococci and almost never occurred prior to or after recovery from inoculation. Muscular spasms developed among patients irrespective of undue concentration of carbon dioxide. Hence, it is suggested

that the neurotoxin produced by the streptococci among patients and animals specifically increased cerebral irritability to such a degree that muscular spasms occurred in the presence of relatively slight acidosis, or even without acidosis, during general anesthesia.

The data obtained are in accord with previous studies (1, 6, 7) and clinical observations (2, 8–13) and indicate that a neurotoxin produced by highly specific neurotropic streptococci during subclinical or clinical infection of the respiratory tract or elsewhere may be a contributory cause of muscular spasms during general anesthesia.

Fortunately, intravenous injection of very small amounts (3 cc.) of 2.5 per cent solution of pentothal sodium usually sufficed to control the spasms of patients permanently. Equivalent injections into rabbits often caused the spasms that had developed after injection of the streptococcus to disappear permanently, if the spasms were slight at the time, and temporarily, if they were severe.

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COMING EXAMINATIONS

The next Written Examinations (Part I) for certification by the American Board of Anesthesiology, Inc., will be held at various places throughout the country on January 19, 1945.

The next Oral Examinations (Part II) of the Board will be held in Philadelphia preceding the scientific assembly of the American Medical Association in June, 1945. Applications must be filed 90 days prior to the date of examination. Secretary: Paul M. Wood, M.D., 745 Fifth Avenue, New York 22, N. Y.