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TREATMENT IN BOTULISM *

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Medical research during the past ten years has greatly advanced our knowledge of *Clostridium botulinum* (*Bacillus botulinus*) and of *botulism*. Recent occurrences of *botulism* in man and domestic animals throughout the United States, and particularly in California, have drawn attention to and greatly stimulated research in all phases of this disease. Until recently these researches were conducted mainly by investigators connected with the national government and state institutions. The National Canners Association is spending large sums of money investigating food poisoning with the object of improving canning methods and preventing further outbreaks of botulism.

E. C. Dickson¹ has energetically attacked the problems connected with the subject of botulism. He has established the common occurrence of botulism in the United States, the fact that toxin is found in preserved fruits and vegetables, the occurrence of two types of toxin in this country, as in Europe, the relation between *C. botulinum* and "limberneck" in chickens and the symptom complex.

G. S. Burke² has established the common occurrence of the organism in nature and the extreme heat resistance and heat inhibition of the spores, thus accounting for the occurrence of the organism and its toxins in preserved foods. She has also designated the two types of *C. botulinus* found in this country as types A and B.³

Robert Graham⁴ and his co-workers have made extensive investigations and have definitely established the relation between *C. botulinum* and forage poisoning.

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1. Dickson, E. C.: Botulism, Monograph No. 8 of the Rockefeller Institute for Medical Research, and subsequent papers.

2. Burke, G. S.: The Occurrence of *B. botulinus* in Nature, *J. Bacteriol.* **4**:541 (Sept.) 1919; The Affect of Heat on the Spores of *B. botulinus*, *J. A. M. A.* **72**:88 (Jan. 11) 1919.

3. Burke, G. S.: Notes on *B. botulinus*, *J. Bacteriol.* **4**:555 (Sept. 5) 1919.

4. Graham, Robert: Studies in Forage Poisoning, *Bulls.* 207 and 208, Kentucky Agricul. Exper. Station, and subsequent papers.

A number of others have verified and extended the results of these workers, so that at the present time we have a fairly satisfactory knowledge of *C. botulinum* and the symptoms produced by its toxins in man and domestic animals.

While our knowledge has been developing along these lines, there has been little or no advance toward a satisfactory treatment in botulism. The Europeans had already shown that an antitoxin could be produced and would neutralize the toxin in "in vitro" and to a limited extent in "in vivo" experiments. Kemper⁵ was able to show that if antitoxin was injected twenty-four hours after the injection of a forty-eight hour fatal dose of toxin, a number of the animals could be saved. And Dickson and Howitt⁶ have verified these findings and shown that when rabbits are fed the toxin in amounts sufficient to cause death in forty-eight hours, the injection of a homologous antitoxin in twenty-four hours will save the animals. No one has, so far as I am aware, fed toxic cultures of *C. botulinum* and then shown that the animals could be saved by the use of an antitoxin after the symptoms appear. This is the condition met with in general practice, and until it is shown that men or animals can be saved from fed lethal doses of toxic cultures of *C. botulinum* by the injection of antitoxin at or about the time that the symptoms appear, we must not look forward with any great expectations to the use of antitoxin in the treatment of botulism.

An examination of the literature offers no encouragement in so far as nonspecific treatment is concerned. We find that physicians have used a wide range of substances in treating cases of botulism. None of these men affirm that the treatment used materially altered or checked the course of the disease.

In viewing the problems presented by our present knowledge of the subject it seemed to us that future research in the treatment of botulism should proceed along four definite lines: (1) to determine the exact nature of the action of the toxin in the body; (2) to determine whether the toxin is extensively produced in the animal body and if produced, to develop some means of preventing its production; (3) to determine the amount of protection to be expected from the use of antitoxin if given at or about the time the symptoms appear; (4) the development of some preliminary treatment which will delay or prevent any further absorption of the toxin pending the arrival of the physician or the antitoxin.

We have no exact knowledge of the nature of the action of the toxin in the body. The result of the action is very evident in loss of

5. Kempner: Weitere Beiträge zur Lehre von der Fleischvergiftung. Das Antitoxin des Botulismus, Ztschr f. Hyg. u. Infektionskrank **26**:481, 1897.

6. Dickson and Howitt: Botulism. A Preliminary Report of a Study of the Antitoxins of *B. botulinus*, J. A. M. A. **74**:718 (March, 13) 1920.

muscular control, death usually resulting from respiratory failure. It has not been determined whether the action of the toxin is central or terminal. The solution of this problem may give us a starting point for the development of some treatment of value.

It is generally stated in the literature that botulism is caused by the ingestion of preformed toxins. Orr⁷ has taken issue with this statement and presents the results of some experiments which suggest that some cases of botulism may be due to an infection. This is important to the physician as well as to the canners. It is obvious that if we are dealing with an infection, the treatment must differ from what it should be if we are dealing only with the ingestion of preformed toxins. This question of infection will be discussed in detail later.

The experience recorded in the use of antitoxin in practical therapy is largely discouraging. There are several reasons for the apparent failure of the antitoxin treatment in botulism, the principal ones being that in most cases a heterologous antitoxin was used, and in all cases the treatment was commenced late in the course of the disease, usually several days after the symptoms appeared. Further experimental work should be done to determine just how effective the antitoxin treatment will be in all stages and conditions of botulism.

Since antitoxin is obtained only after delay, if obtained at all, it is essential that the attempt be made to develop some effective non-specific treatment. Most of the experimental work recorded in this paper was done in an effort to develop a treatment of this type. It was planned to make an exhaustive study of this phase of the subject but unfavorable conditions forced a termination of the work and this preliminary report is presented with the hope of stimulating others to carry on these researches to a definite termination.

An examination of case reports shows that the different treatments used have been designed to stimulate the patient, to prevent further absorption of the toxin and to neutralize the toxin by the use of antitoxin. No one has made any serious attempt to show that the organism or its toxins can be destroyed in the digestive tract. The results obtained from the treatments used are very discouraging. No one ventures to assert that the treatments used have altered the death rate. Dickson⁸ recommends emesis, lavage, purgation with magnesium sulphate or castor oil, irrigation of the colon, giving water by rectum or by hypodermoclysis, strychnin, pilocarpin to counteract diminished secretions, cardiac and other stimulants as needed. Frost⁹ recommends

7. Orr: *Abstr. Bacteriol.* 4:10 (Feb.) 1920.

8. Dickson: *Monograph No. 8*, Rockefeller Institute for Medical Research.

9. Frost: *Am. Med.*, February, 1915, p. 85.

the use of ipecac to stimulate glandular secretions in the intestines to check absorption. He also recommends rectal feeding, heroin, calomel, epinephrin, peptonized milk and whisky. Others have resorted to the use of digitalis, camphor, atropin, sodium phosphate, intravenous injections of salt solution, Glauber's salt, Epsom salt, olive oil, strophanthin, pituitary extract, gelsemium, colon enemas, stomach pump for evacuation and feeding, feeding through duodenal tube, and warm blankets for making the patient more comfortable. In a few cases the patient reported some temporary relief. Pilocarpin gives temporary relief from the accumulation of tenacious mucus in the pharynx. Pneumonia is frequently a complicating cause of death and should always be guarded against.

INFECTION VERSUS PREFORMED TOXINS

The possibility of botulism resulting from the ingestion of toxin-free organisms is discussed here in considerable detail because of its importance to the canning industry, to the epidemiologist, its bearing on the treatment of botulism and because the available data are not conclusive.

After considerable experimental work, von Ermengem¹⁰ concluded that *C. botulinum* does not produce its toxins in the animal body. He fed washed bacilli and also injected them subcutaneously and intravenously without producing symptoms of botulism. He recovered the organism from the spleen and liver but could not demonstrate its presence in sections.

Thom, Edmondson and Giltner¹¹ attempted to determine whether *C. botulinum* spores freed from toxin by heating and by washing with salt solutions would produce toxin within the animal body. They found that if cultures containing spores were freed of toxin by heating to 80 C. for ten minutes and then were fed to or injected into animals no symptoms developed. If the organisms were washed seven times and injected into guinea-pigs or rabbits, the animals died, but if the cells were washed fourteen times the animals lived. In the heated cultures, the bacilli and their toxins were destroyed, leaving only the spores. In the washed cultures, the cells and spores remained, only the toxin being removed. The results obtained from washing the organisms fourteen times seem to indicate that the difference in results between heating and washing seven times is not due to the presence of more cells or to the presence of bacilli as well as spores and the produc-

10. Von Ermengem: Contributions à l'étude des intoxications alimentaires, Arch. pharmacodyn. **3**:213 1897.

11. Thom, Edmondson and Giltner: J. A. M. A. **73**:907 (Sept. 20) 1919.

tion of toxin in the body, but to the presence of preformed toxins which were not washed away. The organisms were recovered from the feces of the animals which were fed spores (freed from toxin either by heat or by washing), and from the liver and spleen of animals injected with cultures. These authors conclude that "colonization of the bacilli in the body has not been proved thus far, but the foregoing isolations from feces prove that the organism goes through the guinea-pig's body in from twenty-four to forty-eight hours with undiminished virulence."

Armstrong, Story and Scott¹² repeated with some modifications the experiments of Thom, Edmondson and Giltner. They injected subcutaneously in guinea-pigs 300,000,000 bacilli, previously heated to 80 C. for thirty minutes. No symptoms developed. The bacilli undoubtedly were killed by the heat, and they do not estimate the number of spores present. Cultures following the heating indicated the presence of viable organisms. Guinea-pigs injected subcutaneously with 300,000,000 bacilli, previously washed twelve and fourteen times, respectively, died. A guinea-pig injected subcutaneously with 120,000,000 organisms which had been washed on a Berkefeld filter by passing 800 c.c. of saline solution through the filter died in four days. Guinea-pigs receiving 12,000,000 similar organisms remained well. A guinea-pig force fed 1,200,000,000 washed organisms died in seventy hours, while another animal given the organisms on grass and meal showed no symptoms. The authors apparently favor the view that it is difficult to wash the toxin from the bacilli and that the death of the guinea-pigs in their experiments was due to this failure rather than that the toxin was produced in the animal bodies. It seems to us that a third explanation is possible, i. e., that there is some intracellular toxin and this is liberated on the death and lysis of the bacilli. This may explain the results obtained in the experiments of Armstrong, Story and Scott, in which a guinea-pig receiving 120,000,000 or more washed bacilli died, while a guinea-pig receiving 12,000,000 similar organisms lived. However, it does not explain the results obtained by Thom, Edmondson and Giltner in which it was shown that a guinea-pig injected with bacilli washed seven times died, while a guinea-pig injected with the same number of bacilli washed fourteen times lived, unless we assume that continued washing extracts the toxin from the interior of the cells.

Orr¹³ has recently recorded some experiments which differ from those already described. All the details of Orr's work are not yet available so that we cannot carefully compare his experiments with the work of others. Orr found that guinea-pigs receiving large numbers of

12. Armstrong, Story and Scott: *Pub. Health Rep.* **74**:2894 (Dec. 19) 1919.

13. Orr: *Abstr. Bacteriol.* **4**:10 (Feb.) 1920.

toxin free spores (heated to 80 C. for thirty minutes) either per os or subcutaneously, usually died in from two to four days. Control pigs receiving antitoxin lived. The organisms were recovered from the spleen and liver in many of the animals, and in one case botulinus toxin was demonstrated in the brain tissue by mouse inoculation. Orr concludes that some cases of botulism may be due to a true infection.

Orr's results differ from those of other workers and suggest that *C. botulinum* may develop and produce its toxins in the animal body. Whether it ever happens that toxin-free organisms are ingested in sufficient numbers to cause botulism is of more interest to the canners and epidemiologists than to the physician. What is of particular interest to the medical profession from the standpoint of treatment is whether the toxin is being produced in the patient after the symptoms appear. The first case requires that the organisms grow and produce toxin in a normal digestive tract; the second, that the organisms grow and produce toxin after peristalsis has been reduced or has ceased. If the first occurs, the second naturally follows, but not the reverse. The production of toxin in the human body, if it occurs at all, may occur only after the ingestion of preformed toxin. Experiments were begun to determine this point, but were not carried far enough to warrant reporting.

If botulism ever occurs in human beings following the ingestion of toxin-free bacilli or spores we should find evidence of it. (1) There should be recorded outbreaks of botulism without a history of spoiled food and (2) without a history of preserved food. (3) It should be possible to demonstrate experimentally an infection following the ingestion of toxin-free organisms. (4) There should be more cases of botulism, unless an extremely large number of organisms are necessary for the development of an infection. (5) Outbreaks of botulism should follow the eating of thoroughly cooked spoiled preserved foods.

1. THERE SHOULD BE RECORDED OUTBREAKS OF BOTULISM
WITHOUT A HISTORY OF SPOILED FOOD

We cannot say that all outbreaks of botulism have been due to spoiled food. Nor can we say that there have been outbreaks of botulism without a history of spoiled food. The records of the early outbreaks are incomplete. In all recent, thoroughly investigated outbreaks it has been stated by some of those involved in each outbreak that the food under suspicion tasted a little "off"; others have stated that they noticed nothing peculiar about the food, and, in fact, liked it.

This criterion is of little value in helping us to reach a decision because of the fact that spoiled foods are not readily separated from

unspoiled foods, and that people are careless about discarding food that is slightly suspicious. The standards by which spoilage in food is judged differ with different people, and food containing a naturally strong or peculiar odor may be in the early stages of botulinus spoilage and contain toxin sufficiently strong to cause disease without the taste, smell or appearance being sufficiently altered to attract the attention of the average individual. Also when mild flavored, slightly decomposed food containing toxin is mixed with other foods the detection of any unusual condition of the food is difficult. Such foods would be classed as unspoiled by some individuals. Some scientific observers believe that botulinus spoilage in preserved foods can always be detected by a careful examination, others are doubtful. The subject of botulism has received such publicity that we may expect to see a reduction in the number of outbreaks in families where the housewife oversees the cooking, but in many institutions and public eating houses the ignorant cook still rules. For this reason, the evidence given as to the condition of the suspected food will in some cases be inconclusive and of little value in determining whether there are ever any outbreaks of botulism without a history of spoiled food.

2. THERE SHOULD BE RECORDED OUTBREAKS OF BOTULISM
WITHOUT A HISTORY OF PRESERVED FOOD

It has been shown that *C. botulinum* occurs in nature on fresh fruits and vegetables. It follows that many of us ingest the organisms without ill effects. If the ingestion of small numbers of toxin-free spores causes an infection, we should have cases of botulism without a history of preserved food having been eaten a short time before the onset of the symptoms. The epidemiologic data concerning most of the outbreaks of botulism are not sufficiently detailed to prove without question that a certain food caused the outbreak. But the fragmentary evidence that we have indicates that all outbreaks of botulism have been preceded by the eating of preserved food. It has not been shown in the early outbreaks of botulism that these preserved foods contained the toxin or the organism of *C. botulinum*. However, in all recent thoroughly investigated outbreaks it has been shown that the preserved foods eaten, if any remained for investigation, contained *C. botulinum* or its toxin. Therefore, we feel justified in believing that botulism in man does not follow the ingestion of toxin-free spores, particularly in small numbers. We have no evidence that large numbers of toxin-free spores are ever ingested, except in cooked spoiled food. And the destruction of the toxin in spoiled foods by thorough boiling for a few minutes does not destroy the spores but does appear to prevent botulism.

3. IT SHOULD BE POSSIBLE TO DEMONSTRATE EXPERIMENTALLY
AN INFECTION FOLLOWING THE INGESTION OF
TOXIN-FREE ORGANISMS

We have already cited the results obtained by von Ermengem, Thom, Edmondson and Giltner, and Armstrong, Story and Scott, in which infections did not follow the feeding and injecting of toxin-free organisms, and of Orr, who was able to demonstrate the presence of toxin in the animal body following the feeding of toxin-free spores. The conflicting results obtained by these workers have not been explained, and we must await repeated experiments for knowledge of the determining factors. Orr's results suggest that an infection may sometimes follow the ingestion of large numbers of toxin-free spores.

THERE SHOULD BE MORE CASES OF BOTULISM UNLESS AN EXTREMELY
LARGE NUMBER OF ORGANISMS ARE NECESSARY FOR
THE DEVELOPMENT OF AN INFECTION

As has already been stated, the organisms are fairly common in nature, and many of us ingest them without the development of an infection. That there are not more cases of botulism indicates that a few toxin-free organisms will not start an infection in a normal individual. The experimental evidence shows that an infection following the ingestion or injection of toxin-free spores does not occur unless very large doses are given. We cannot conceive of any condition under which a very large number of toxin-producing organisms of *C. botulinum* would be ingested, except in the eating of heated preserved foods. Orr has reported strains of *C. botulinum* as having lost the power of toxin production. Such strains growing in preserved foods might afford an opportunity for the ingestion of toxin-free organisms. Since the virulence of the organism depends partly on toxin production, it is a question whether such strains, even if taken in large numbers, would regain the power of toxin production and cause an infection.

The statistical and experimental evidence indicates that botulism does not follow the ingestion of small numbers of toxin-free organisms. Whether toxin-free organisms ever occur in preserved foods, heated or unheated, in sufficiently large numbers to cause an infection in man is doubtful.

THERE SHOULD BE CASES OF BOTULISM FOLLOWING THE EATING OF
THOROUGHLY COOKED SPOILED PRESERVED FOODS

No cases of botulism are recorded in this country as following the eating of thoroughly cooked food. We assume that much spoiled food has been cooked and eaten. Outbreaks are reported in which the eating of uncooked spoiled food caused botulism and the eating of portions

of the same food after cooking caused no symptoms. This is not entirely conclusive since occasionally a person eating the uncooked food also escapes illness. At least seven individuals have been reported as having eaten of food causing botulism in others without becoming ill.

Since there are no recorded outbreaks of botulism in this country following the eating of cooked spoiled food, and since the spores are highly resistant to heat, we believe it reasonable to assume that large numbers of spores have been eaten without causing symptoms of botulism.

It is to be expected, however, that cases of botulism will occasionally occur from the eating of insufficiently cooked or heated spoiled food. A little warming will not destroy all the toxin. Spoiled food containing considerable gas appears to be boiling at temperatures of from 70 to 80 C. Bubbles may rise to the surface for at least five minutes before the true boiling point is reached. The statement is found in the literature that boiling spoiled food for five minutes makes it safe to eat. Thom, Edmondson and Giltner reported that at a temperature of 75 C. no appreciable time is required for the destruction of the toxin in a filtrate. Orr records destruction of the toxin in two minutes at 80 C. Others have reported similar findings. Boiling ordinary canned fruits and vegetables for five minutes should raise the temperature in the fruits or vegetables up to or above 75 C. and cause the destruction of all the toxin present, unless the toxin in the fruit tissues and juices is more resistant to heat than toxin in filtrates. In foods in which there is so much gas present that boiling appears to begin at 75 C., an exposure to this temperature for five minutes may not destroy all the toxin in the interior of the food, and botulism may result from the eating of such cooked food and be caused by the undestroyed toxin.

Experiment to determine the effect of heat on the disease producing power of the juice from a can of spoiled beans containing C. botulinum and its toxin.—The following experiments throw some light on the questions here involved and they simulate as nearly as possible conditions found in home cooking. The beans used were canned six months previously and contained a strong Type A toxin. The beans were badly spoiled and contained gas. One half pint of the beans with the juice was placed in a large beaker and brought to a boil. At intervals some of the juice was withdrawn and injected subcutaneously into guinea-pigs. The results obtained (Table 1) indicate that:

1. Spoiled foods containing gas may appear to be boiling for several minutes before the true boiling point is reached.
2. The destruction of the toxin in the bean juice may require considerable more exposure to heat than is recorded for toxin in filtrates.

3. Under the conditions of this experiment the disease-producing power of the bean juice was not wholly destroyed after exposure for nine minutes to temperatures changing from 70 C. to 100 C. or after exposure to boiling for four minutes.

4. The disease producing power of a can of spoiled beans containing *C. botulinum* and its toxin may be destroyed by a temperature that does not destroy the spores of *C. botulinum*.

5. Spoiled canned foods may appear to have boiled seven minutes and still be unsafe to eat.

TABLE 1.—EFFECT OF HEAT ON THE DISEASE PRODUCING POWER OF JUICE FROM SPOILED BEANS CONTAINING *C. BOTULINUM* AND ITS TOXIN

Time Minutes	Temperature and Appearance of Beans	Guinea- Pig	Weight, Injected, Gm.	Amount C.c.	Results
	Unheated.....	1	250	1	Dead 18 hours
	Unheated.....	2	250	0.01	Dead 18 hours
0	Burner lighted				
4	70 C. bubbles beginning to rise				
6	82 C. active bubbling, resembling boiling				
7	92 C. active bubbling, resembling boiling.....	3	250	1	Dead 96 hours
8	98 C. large bubbles				
9	100 C. true boiling.....	4	250	1	Dead 96 hours
13	100 C. true boiling.....	5	250	1	Dead 17 days
19	100 C. true boiling.....	6	250	1	Lived

In this experiment no effort was made to determine whether the animals died from the effects of the toxin formed in the can of beans or in the animal body. A filtrate of the heated juice was not tested for toxin and the presence of spores was not determined. However, one fact of importance is evident. The disease producing power of a can of spoiled beans may be destroyed by a temperature that does not destroy the spores of *C. botulinum*. Exposure of the spores to a temperature of 100 C. for ten minutes does not prevent their development and toxin production in culture mediums. If the spores ever cause an infection, heat may affect their virulence for the animal body. This experiment cannot serve as the basis for any definite conclusions as regards the infection theory because of the three unknown and uncontrolled factors: (1) the effect of heat on the toxin in bean juice; (2) the effect of heat on the vegetative forms in bean juice; (3) the presence of spores and the effect of heat on the virulence of the spores of *C. botulinum*. The results indicate, however, that we may expect to have outbreaks of botulism following the eating of poorly cooked spoiled food.

The results of this experiment indicate that spoiled food to be safe to eat must be boiled for at least fifteen minutes. And if the spores of *C. botulinum* are found to cause an infection in man, a much greater exposure to heat may be required. Boiling for four hours does not destroy all the spores of *C. botulinum*.

Experiment to determine the effect of boiling on the disease producing power of the juice from a can of spoiled beans containing C. botulinum and its toxin when oil stratified.—About 100 c.c. of the beans and juice used in the preceding experiment were placed in a beaker, oil stratified, and exposed to heat at 100 C. The results obtained (Table 2) were similar to those of the preceding experiment, except that the disease producing power of the bean juice resisted the effects of the heat for a longer period of time. Guinea-pigs receiving 1 c.c. of the bean juice, exposed to 100 C. for ten minutes, died.

TABLE 2.—EFFECT OF TEMPERATURE OF 100 C. ON THE DISEASE PRODUCING POWER OF JUICE FROM SPOILED BEANS CONTAINING C. BOTULINUM AND ITS TOXIN WHEN OIL STRATIFIED

Time, Minutes	Temperature	Amount of Juice Injected, C.c. Guinea-Pig		Weight, Gm.	Result
	Unheated	1	1	250	Dead, 18 hours
	Unheated	0.01	2	250	Dead, 18 hours
0	Burner lighted				
6½	100 C.				
11½	100 C.	1	3	250	Dead, 96 hours
16½	100 C.	1	4	250	Dead, 96 hours
21½	100 C.	1	5	250	Lived
26½	100 C.	1	6	250	Lived

Experiment to determine the effect of a temperature of 80 C. on the disease producing power of a can of spoiled beans, containing C. botulinum and its toxin, when oil stratified in a test tube and placed in a water bath.—About 15 c.c. of the beans and juice used in the preceding experiments were placed in a test tube and oil stratified. This test tube was then placed in a water bath and kept at 80 C. No record was taken of the temperature in the test tube. Some of the bean juice

TABLE 3.—EFFECT OF TEMPERATURE OF 80 C. ON THE DISEASE PRODUCING POWER OF JUICE FROM SPOILED BEANS CONTAINING C. BOTULINUM AND ITS TOXINS WHEN OIL STRATIFIED IN A TEST TUBE AND PLACED IN A WATER BATH

Time, Minutes	Temperature	Amount of Juice Injected, C.c. Guinea-Pig		Weight, Gm.	Result
	Unheated	1	1	250	Dead, 18 hours
	Unheated	0.01	2	250	Dead, 18 hours
0	Test tube containing beans placed in water bath				
11	80 C.	1	3	250	Dead, 72 hours
30	80 C.	1	4	250	Dead, 72 hours
61	80 C.	1	5	250	Dead, 72 hours

was taken from the test tube at intervals and injected into guinea-pigs. The results obtained (Table 3) indicate that under the conditions of this test, which largely resembles the conditions surrounding beans in the middle of a kettle placed on the stove to warm, the disease producing power of the bean juice was not destroyed after an exposure to a temperature of 80 C. for one hour. This suggests that the casual warming of spoiled foods to a temperature of 80 C., even over an

extended period of time may not make them safe to eat. Since such foods may appear to be boiling, it is evident that we may expect to have cases of botulism following the eating of so-called boiled foods. The epidemiologist must bear these facts in mind in investigating outbreaks of botulism.

INFECTION FOLLOWING THE INGESTION OF THE TOXIN AND THE ORGANISMS

While it is of great importance to the epidemiologist to know whether botulism ever follows the ingestion of toxin-free organisms, it is of equal importance to the physician to know whether an infection ever occurs following the ingestion of the toxin and the organisms. If botulism ever occurs following the ingestion of toxin-free spores, we may be justified in assuming that an infection may occur following the ingestion of both the toxin and the spores, particularly after the symptoms appear. In the latter case, conditions for the development of *C. botulinum* are presumably more favorable than in the normal digestive tract.

If an infection ever occurs this may account for relapses that sometimes follow an improvement in the condition of the patient. It sometimes happens that a patient shows considerable improvement and then suffers a relapse and dies. This may be accounted for on the basis of a required incubation period, the first symptoms being due to the ingested toxin and the relapse due to toxins produced in the body. Stiles,¹⁴ in recording his own case states that he had a serious relapse after partially recovering from the first attack. Others have reported similar experiences.

If an infection occurs, i. e., production of toxin in the digestive tract or elsewhere in the animal body, following the ingestion of the toxin and the organisms of *C. botulinum* the following postulates should hold true.

1. There should be, within certain limits, no relation between the amount of infected food eaten and the severity of the symptoms. If infection does not occur then there should be a definite relation between the amount of food eaten and the severity of the symptoms. (Individual variations in resistance, effects of other foods eaten and the even distribution of the toxin in the food are modifying factors that must be taken into consideration.)

Armstrong, Story and Scott,¹⁵ in investigating the Canton, Ohio, outbreak found a "remarkable correspondence between the amount

14. Stiles: J. A. M. A. **61**:2301 (Dec. 27) 1913.

15. Armstrong, Story and Scott: Pub. Health Rep. **34**:2877 (Dec.) 1919.

eaten and the severity of the illness.” Those eating less of the poisonous food obtained less toxin and also a smaller number of organisms but “had the bacilli swallowed with the olives been capable of growing and producing toxin in the alimentary tract it seems that some of the people who ate small amounts but were little affected would have developed serious symptoms.” They estimated that one olive contained several million bacilli and conclude that “a bite of olive containing this number of viable organisms, if capable of multiplying and forming toxin in the alimentary tract, would have caused serious infections.” However, this does not show that an infection may not occur following the ingestion of large numbers of organisms and after paralysis has set in.

2. It should be possible to demonstrate the infection experimentally since the M. L. D. (minimum lethal dose) of the filtrate should be less than the M. L. D. of the culture.

Any difference in toxicity between filtrate and culture should be due to the organisms present in the culture unless the process of filtering eliminates some of the toxin as well as the organisms. Emerson and Collins¹⁶ report that olive juice from spoiled olives “filtered through a Berkefeld filter was found to be slightly less toxic than the unfiltered juice.” We await further experimental evidence of a similar nature.

3. If infection occurs in the body outside of the digestive tract it should be possible to detect the organisms in sectioned material. *C. botulinum* has been recovered at necropsy from the liver and spleen of both human and laboratory animals dead of botulism. If the organisms are able to multiply and start an infection in the spleen and other organs it should be possible to demonstrate their presence in sectioned material. All attempts to demonstrate the organism in microscopic sections of organs of animals dead of botulism have been unsuccessful.

CONCLUSION

The major evidence as we have presented it here favors the view that botulism in man very rarely, if ever, occurs following the ingestion of toxin-free organisms. The available evidence is too inconclusive to form the basis for any definite conclusions. Many of the facts presented can be explained in more than one way. Our own belief is that infection in human beings following the ingestion of toxin-free organisms never occurs. We are inclined to believe that the organism does produce toxin in the alimentary tract following the ingestion of preformed toxins and after paralysis has set in. Treatment

16. Emerson and Collins: J. Lab. & Clin. Med. 5:559 (June) 1920.

of the digestive tract should be designed to neutralize and wash out the toxin and inhibit or destroy the organisms.¹⁷

IMMUNE SERUM TREATMENT

Workers in Europe and in this country have shown that repeated injections of sublethal doses of *C. botulinum* toxin in various animals will stimulate the production of specific antitoxins. Experiments have shown that the antitoxin will neutralize the toxin in "in vitro" experiments and in "in vivo" if the antitoxin is injected before the toxin, or after the toxin if the injection is made before the system is fatally damaged. Since there are at least two immunologically distinct types of *C. botulinum*, it is necessary to use a polyvalent immune serum unless the type of toxin is known.

The extent to which antitoxin is effective in preventing death when injected some time after the toxin has been taken into the body has not been established definitely. Kempner²⁰ has shown that if antitoxin is injected twenty-four hours after a forty-eight hour fatal dose of

17. After the present article was sent to press our attention was called to an article by Edmondson, Giltner and Thom (The Possible Pathogenicity of *Bacillus Botulinus*, Arch. Int. Med. **26**:357 [Sept.] 1920), in which they discuss experiments the results of which agree with those of Orr. In referring to their experiments they state that, "They show that occasionally guinea-pigs will develop the specific disease and die when fed or injected with large doses of the spores freed from toxin in routine manner," i. e., by washing or heat or both. The authors contemplate experiments to determine whether the toxin causing the disease is present in the spores or bacilli when ingested or injected and protected from heat by the spore wall and released upon dissolution of the spore wall or is actually produced in the body after the spores germinate. One of the strains used caused death more consistently than the other. The feeding or injection of large numbers of organisms of the most virulent strain was not always fatal. One of the body defenses against anaerobe infection consists of the amount of oxygen in blood and tissue fluids. The injection of toxin free spores either subcutaneously or intravenously, may lead to a local lowering of the oxygen tension and necrosis making conditions favorable for the development of anaerobes. What factor this plays in experimental botulism remains to be determined. *C. botulinum* has not been recorded from war wounds. The experimental data suggests that it may occasionally occur in wounds and thus account for the failure of the patient to respond to tetanus and other common antitoxin treatment. The paper of Bullock and Cramer¹⁸ suggests that the toxin free spores of tetanus are no more infectious than those of *C. botulinum*. The main problem from the standpoint of treatment is to determine whether there are outbreaks of botulism in man following the ingestion of toxin free spores and if such do occur whether the symptoms are typical or atypical as has been suggested by Shippen¹⁹ and therefore leading to a wrong diagnosis.

18. Bullock and Cramer: A New Factor in the Mechanism of Bacterial Infection, Proc. Roy. Soc. London, Series B **90**:513, 1920.

19. Shippen: Arch. Int. Med. **23**:346 (March) 1919.

20. Kempner: Weiterer Beitrag zur Lehre von der Fleischvergiftung. Das Antitoxin des Botulismus, Ztschr. f. Hyg. u. Infektionskrankh. **26**:481, 1897.

toxin many of the animals will live. Dickson and Howitt⁶ verified Kempner's findings to the extent of showing that if the antitoxin is injected subcutaneously within twenty-four hours after a forty-eight hour fatal dose of toxin has been ingested the animals will live. This means that when small doses of toxin are fed, twenty-four hours is not sufficient time for the toxin to be absorbed and cause fatal injury to the animal. Antitoxin must be used before the nervous system has been injured fatally. The time required for this depends upon the amount of toxin ingested or injected. If large amounts of toxin are used an animal may be injured fatally by the time the first symptoms appear.

The problem the practitioner has to face during an outbreak is to save the patients showing symptoms and also those who have eaten the poisonous food but have not yet shown symptoms. No one has, so far as we are aware, shown experimentally that an animal can be saved from lethal doses of toxin by the injection of antitoxin after the symptoms appear. And no one has attempted to show how many hours before the symptoms appear the injection of antitoxin will protect. The experiments of Kempner and of Dickson show that an animal can be saved from a forty-eight hour fatal dose of toxin by the injection of antitoxin twenty-four hours before death would occur if the antitoxin was not injected. They do not indicate how many hours this would be before the symptoms appeared. Dickson injected the antitoxin subcutaneously. It might have been possible to protect his animals by later injections if these had been made intravenously.

The available statistical data of the various outbreaks in this country show a death rate of 90 per cent. among those first showing symptoms, and a death rate of 60 per cent. among those last showing symptoms. Frequently, two or three days will elapse between the appearance of symptoms in the first patient and the last patient to come down in an outbreak of botulism. Sixty per cent. of the latter die. The experimental evidence gives us reason to believe that some of these victims could have been saved by the injection of antitoxin if the injections had been begun as soon as the first patient showed symptoms.

The following experiments were designed to determine the extent of the protection afforded by antitoxin if injected after the symptoms appear. In order to approach the conditions found in general practice toxic cultures instead of toxic filtrates were used.

Experiment to determine whether rabbits can be protected from lethal doses of toxic cultures of C. botulinum by the injection of antitoxin as soon as the symptoms appear.—Each rabbit was force fed 6 c.c. of a ten day meat culture of *C. botulinum* Type B. Antitoxin was injected both subcutaneously and intravenously seventeen hours later.

At this time two of the rabbits were dead, one was practically dead and three showed slight symptoms of prostration. Two of the rabbits receiving antitoxin lived. Since these rabbits received about 2 minimum lethal doses of toxin and were showing the first symptoms of botulism, there is no question that they would have died without the injections of antitoxin. The results obtained (Table 4) indicate that some rabbits can be saved from the forced feeding of a lethal amount of a toxic culture of *C. botulinum* by the injections of antitoxin as soon as the symptoms appear. It is probable that a larger percentage of rabbits can be saved by the injection of antitoxin after the symptoms appear if a smaller amount of toxin is fed.

TABLE 4.—RESULTS OF AN EXPERIMENT TO DETERMINE WHETHER RABBITS CAN BE SAVED FROM LETHAL DOSES OF TOXIC CULTURES OF *C. BOTULINUM* WHEN FORCE FED BY INJECTION OF ANTITOXIN AS SOON AS SYMPTOMS APPEAR

Rabbit	Weight, Gm.	Amount of Culture	Interval Before Injection of Antitoxin	Results
1	1,750	6 c.c. per os		Dead, 16 hours
2	2,700	6 c.c. per os		Dead, 16 hours
3	2,850	6 c.c. per os		Dead, 17 hours
4	1,925	6 c.c. per os	17 hours	Lived
5	2,115	6 c.c. per os	17 hours	Lived
6	2,750	6 c.c. per os	17 hours	Dead, 23 hours

The experiment was repeated with the exception that the injection of the antitoxin was delayed until the symptoms were more advanced.

Experiment to determine whether rabbits can be saved from lethal doses of toxic cultures of C. botulinum by the injection of antitoxin after the symptoms are well advanced.—Each rabbit was force fed 6 c.c. of the meat culture used in the preceding experiment. The injections of antitoxin were begun twenty-one hours after the feeding of the toxic cultures and were made subcutaneously and intravenously. At this time three of the rabbits were dead and the three receiving antitoxin were prostrated. All the rabbits receiving antitoxin died. One of these lived for sixty hours, but it cannot be certain that this was due to the action of the antitoxin, as rabbits show considerable variation in their resistance to the toxin when force fed. The results obtained (Table 5) indicate that rabbits cannot be saved from 2 M. L. D., force fed, of a toxic culture of *C. botulinum* by the injection of antitoxin after the symptoms are well advanced and the rabbits show evident signs of prostration.

Botulinus antitoxin has been used in a number of outbreaks of botulism with inconclusive results. Dickson²¹ was the first in this country to use antitoxin as a therapeutic agent in cases of botulism.

21. Dickson: Arch. Int. Med. **22**:483 (Oct.) 1918.

He used a Type A and B antitoxin and both patients recovered. The antitoxin was not injected until five days after the symptoms appeared, and he doubts whether it affected the course of the disease.

McCaskey²² injected antitoxin in treating three patients in the Decatur outbreak and believed it to be the determining factor in the recovery of two of the patients. The third patient was almost dead at the time the antitoxin was administered. The antitoxin was not given until several days after the symptoms appeared.

Ransom²³ reports the use of antitoxin in the Clinton prison outbreak with beneficial results though given late in the course of the disease.

Randell,²⁴ Jennings, Haass and Jennings,²⁵ Armstrong, Story and Scott, and Sisco²⁶ report the use of antitoxin in treating patients suffering with botulism. No improvement in the condition of the patients was attributed to the use of antitoxin.

TABLE 5.—RESULTS OF AN EXPERIMENT TO DETERMINE WHETHER RABBITS CAN BE SAVED FROM LETHAL DOSES OF TOXIC CULTURES OF *C. BOTULINUM* WHEN FORCE FED BY INJECTION OF ANTITOXIN AFTER SYMPTOMS ARE WELL ADVANCED

Rabbit	Weight	Amount of Culture	Interval Before Injection of Antitoxin	Results
1	1,750	6 c.c. per os		Dead, 21 hours
2	1,950	6 c.c. per os		Dead, 18 hours
3	2,100	6 c.c. per os		Dead, 12 hours
4	2,000	6 c.c. per os	21 hours	Dead, 24 hours
5	1,900	6 c.c. per os	21 hours	Dead, 60 hours
6	2,100	6 c.c. per os	21 hours	Dead, 36 hours

In all cases in which antitoxin has been used in the treatment of botulism, the antitoxin has been given late in the course of the disease, sometimes in small amounts, or was of the heterologous type. Under such conditions we could not expect to get encouraging results.

Experimental work has shown that antitoxin to be effective must be given in excess before or very soon after the symptoms appear. Since a rapid determination of the type of organism producing the toxin is not possible, a polyvalent serum should be used. And since there is a possibility of *C. botulinum* producing its toxin in the body, the serum should be bacteriolytic or bactericidal as well as antitoxic. The serum should be injected intravenously. We have reason to hope that if antitoxin becomes generally available and is given to all those partaking of the suspected food as soon as the first patient shows symptoms, the death rate will be reduced materially.

22. McCaskey: Am. J. M. Sc. **158**:57 (July) 1919.

23. Ransom: Personal communication.

24. Randell: J. A. M. A. **75**:33 (July 3) 1920.

25. Jennings, Haass and Jennings: J. A. M. A. **74**:77 (Jan. 10) 1920.

26. Sisco: J. A. M. A. **74**:516 (Feb. 27) 1920.

EFFECT OF FOODS ON THE TOXIN AND DEATH RATE

A review of available literature brought out certain facts which suggested to us that the foods in the stomach may have an influence on the appearance of the symptoms and the death rate.

The death rate in Europe is from 25 to 30 per cent. less than the death rate in the United States. This difference in the death rate may be due to a number of factors. The principal ones are the following:

1. The outbreaks in Europe are mainly due to toxin formed in foods such as meats, sausage and cheese, which are usually not preserved in liquids. The outbreaks in the United States have usually been due to toxins formed in foods preserved in liquids, such as fruits and vegetables. It is possible that the organism produces a stronger toxin in the latter foods; or that the liquid facilitates absorption.

2. Different habits in the heating and cooking of foods may reduce the death rate. Heat destroys the toxin.

3. The outbreaks in Europe may be due to a different type of *C. botulinum*, or to a prevalence of Type B, which usually produces a weaker toxin than Type A. The majority of the outbreaks in the United States have been produced by the toxin of Type A.

4. The Europeans use more oils and alcoholic drinks with meals. These may affect the rate of absorption or the strength of the toxin.

5. Botulism is not so well known as a specific disease in this country, and it is possible that only the fatal outbreaks have been recognized and reported.

The following facts have a bearing on the question concerning the effects on the toxin of food in the stomach. Occasionally, a person eating the toxic food escapes illness. The appearance of the symptoms in persons eating the same amount of the toxic food varies in time and severity. Variations in the appearance of the symptoms and the mortality may be due to the toxin not being evenly distributed through the food, to individual variation in resistance and to the effect of other foods taken into the stomach at the same time. Armstrong, Story and Scott¹⁵ report that in the Canton, Ohio, outbreak two of those eating heavily of the toxic food escaped with slight symptoms and attribute this to the whisky taken with the meal. They were able to demonstrate that alcohol neutralizes the toxin when the two are mixed in a test tube. In the Boise, Idaho, outbreak one of those eating heavily of the toxic food escaped illness. Van Ermengem states that the toxin is precipitated by alcohol, tannin and neutral salts, and that 20 per cent. normal soda destroys it in a short time. Brieger and Kempner²⁷ state that the toxin in dried form is susceptible to alcohol,

27. Brieger and Kempner: Beitrag zur Lehre von der Fleischvergiftung, Deutsch. med. Wchnschr. **23**:521, 1897.

ether and oxidizing agents. Forsman²⁸ reports that large doses placed in the large intestine of the rabbit produce no symptoms. The M. L. D. "per os" for rabbits is at least 300 times the subcutaneous M. L. D. These facts led us to investigate the effect of the stomach contents and various foods on the toxin of *C. botulinum*. We did not determine the effect of the contents of the intestines on the toxin or the potency of the toxin when introduced into the intestines.

Experiment to determine the neutralizing action of the stomach contents of a normal rabbit on the toxin of C. botulinum.—The contents of a full rabbit stomach was thoroughly dried in the incubator, then ground up in a mortar, mixed with an equal volume of diluted toxin and incubated two hours. The toxin used as a control was incubated at the same time. The toxin-stomach contents mixture was then centrifuged and the supernatant fluid injected subcutaneously into guinea-pigs. The results obtained (Table 6) indicate that the stomach contents of the rabbit do not neutralize or precipitate the toxin of *C. botulinum*.

TABLE 6.—RESULTS OF NEUTRALIZING ACTION OF STOMACH CONTENTS OF NORMAL RABBIT ON TOXIN OF *C. BOTULINUM*

Guinea-Pig	Amount Toxin	Results
Control 1	2 M. L. D.	Dead, 20 hours
Control 2	2 M. L. D., centrifuged toxin.....	Dead, 29 hours
Control 3	2 M. L. D., centrifuged toxin.....	Dead, 29 hours
4	2 M. L. D., toxin exposed to stomach contents.....	Dead, 29 hours
5	2 M. L. D., toxin exposed to stomach contents.....	Dead, 29 hours

The following experiment was designed to determine the comparative rates of absorption of the toxin in the full stomachs and starved stomachs of rabbits. Although the contents of a full rabbit stomach did not neutralize the toxin it was thought that it might affect the appearance of the symptoms and the death rate by mechanically slowing down the rate of absorption.

Experiment to determine the effect of starving and the subsequent empty stomach on the rate of absorption of the toxin in the rabbit.—Two rabbits were given water but no food for forty-eight hours. They were then given a small nibble of grass and force fed 4 c.c. of a toxic filtrate of *C. botulinum* Type B. The grass was fed to stimulate gastric secretion. Two normal rabbits received the same amount of toxin. The starved rabbits died before the controls. Starving the rabbits lowered their resistance to the toxin, or absorption of the toxin was more rapid in the empty stomachs. No experiments were made to determine which factor predominates. If the latter explanation is the

28. Forsman: Beiträge zur Kenntnis der Bakteriologie des Botulismus, Lunds Universitets Arsskrift, 1900; Abstr. Centralbl. f. Bakteriologie, 29:541, 1901.

true one then the amount of nontoxic food eaten by those at meals causing outbreaks of botulism may account for some of the variation in time of the appearance of the symptoms in the patients. The gastric juice apparently has no effect on the toxin. The results obtained (Table 7) suggest that in some outbreaks of botulism the amount of nontoxic food eaten may account for some of the variation in the time of the appearance of the symptoms in the victims and the degree of illness.

TABLE 7.—RESULTS OF STARVING AND SUBSEQUENT EMPTY STOMACH ON RATE OF ABSORPTION OF TOXIN IN RABBIT

Rabbit	Amount Toxin	Condition of Stomach	Results
1	4 c.c.	Full stomach.....	Dead in 144 hours
2	4 c.c.	Full stomach.....	Dead in 48 hours
3	4 c.c.	Starved 48 hours.....	Dead in 35 hours
4	4 c.c.	Starved 48 hours.....	Dead in 18 hours

Experiments were made to determine the effect of different human foods on the toxin of *C. botulinum*. It was thought that some foods might be more effective than others in slowing down the rate of absorption of the toxin. These experiments were designed to determine if the various foods afforded protection against the action of the toxin, if this protection was as great in the stomach as in subcutaneous injections and whether the protection was due to a neutralization of the toxin or to a slowing down of the rate of absorption.

FATS AND OILS

Kempner and Schepilewsky²⁹ state that butter and emulsified oil neutralize about twice the lethal dose of toxin for guinea-pigs when the fat and toxin are mixed and injected subcutaneously. McCaskey urges the use of "castor oil or some other laxative fat, for the double purpose of clearing out the ingested toxin, which is largely formed under saprophytic conditions, and of lowering or destroying the virulence by combination of fats." In consideration of these statements it seemed advisable to determine the extent of the protection afforded by the use of fats, the nature of this protection and if the protection afforded by different fats varied. Typical animal, vegetable and mineral fats and oils were used. The experiments described here are rather limited but the results obtained furnish the basis for certain conclusions which will be given under each experiment.

OLIVE OIL

Experiment to Determine Whether Olive Oil Protects Against the Action of the Toxin of C. Botulinum.—Different amounts of olive oil

29. Kempner and Schepilewsky: Ueber antitoxische Substanzen gegenüber dem Botulismusgift, Ztschr. f. Hyg. **27**:213, 1898.

and a Type A toxin filtrate were used. The oil and toxin were placed in test tubes, emulsified by vigorous shaking and incubated one hour. The tubes were shaken several times during the incubation period. Injections were made subcutaneously into guinea-pigs. The results obtained (Table 8) show that guinea-pigs are protected from the action of the toxin of *C. botulinum* when the toxin and oil are emulsified in a test tube, incubated one hour and injected subcutaneously. Three c.c. of the oil protected against the action of 3 M. L. D. of the toxin but not against 4 M. L. D.

TABLE 8.—EFFECT OF OLIVE OIL AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE EMULSIFIED, INCUBATED AND INJECTED SUBCUTANEOUSLY?

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Olive Oil	Results
1	275	3 M. L. D.		Dead, 20 hours
2	315	3 M. L. D.	3 c.c.	Lived
3	310	3 M. L. D.	6 c.c.	Lived
4	300	4 M. L. D.	3 c.c.	Dead, 70 hours
5	300	5 M. L. D.	5 c.c.	Dead, 96 hours

Experiments were made to determine whether the protection afforded by the olive oil was due to a neutralization of the toxin or to a slowing down of the rate of absorption. We assumed that if the protection afforded by the olive oil is due to a purely physical slowing down of the rate of absorption the following statements would prove to be true.

1. If the mixture, after incubation, is centrifuged the toxin in the water should not show any appreciable reduction in strength.
2. Emulsifying the toxin oil mixture several seconds should be just as effective as incubating several hours.
3. In intraperitoneal injections the protection afforded by the oil would be much less than that in subcutaneous injections and not such as could be accounted for by the difference in the M.L.D. for the two types of injections, and those receiving the oil-toxin mixture should die almost as soon as the controls. In intraperitoneal injections the rate of absorption is much greater.

Experiment to determine whether the strength of the toxin of C. botulinum is reduced after exposure to olive oil.—The same toxin and olive oil were used as in the preceding experiment. The toxin and olive oil were emulsified, incubated two hours and then centrifuged until the oil was free of water. The water was injected subcutaneously in guinea-pigs. The results obtained (Table 9) indicate that the strength of the toxin is affected slightly if at all by exposure to olive oil.

The experiment was repeated, with the exception that the injections were made intraperitoneally. The results obtained agree with those in the preceding experiment. The toxin control pig was dead in fifteen hours; the exposed toxin pig was dead in twenty-four hours and the mixture control pigs were dead in eighty-eight hours.

TABLE 9.—EFFECT OF OLIVE OIL ON THE STRENGTH OF THE TOXIN OF *C. BOTULINUM*

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Oil	Remarks	Result
1	365	3 M. L. D.		Toxin control.....	Dead, 20 hours
2	300	3 M. L. D.	6 c.c.	Mixture control.....	Lived
3	315	3 M. L. D.	3 c.c.	Mixture control.....	Lived
4	325	3 M. L. D.	0 c.c.	Exposed toxin.....	Dead, 40 hours

Experiment to determine whether emulsifying the toxin and olive oil and injecting immediately is just as effective in protecting against the action of the toxin as incubating two hours before injecting.—The same toxin and olive oil was used as in the preceding experiment. The toxin and olive oil were placed in a test tube, shaken vigorously for 30 seconds and then injected subcutaneously into guinea-pigs. The control mixture was incubated two hours. The results obtained (Table 10) indicate that the incubation does not increase the protection afforded by the olive oil.

TABLE 10.—RESULTS OF EXPERIMENT TO DETERMINE WHETHER EMULSIFYING TOXIN AND OLIVE OIL AND INJECTING IMMEDIATELY IS JUST AS EFFECTIVE IN PROTECTING AGAINST ACTION OF TOXIN AS INCUBATING TWO HOURS BEFORE INJECTING

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Oil	Remarks	Result
1	365	5 M. L. D.		Toxin control.....	Dead, 20 hours
2	275	5 M. L. D.	6 c.c.	Mixture incubated.....	Dead, 46 hours
3	265	5 M. L. D.	6 c.c.	Mixture not incubated.....	Dead, 56 hours
4	330	5 M. L. D.	6 c.c.	Mixture not incubated.....	Dead, 56 hours

The results of the above experiment with the olive oil convinced us that olive oil does not neutralize the toxin of *C. botulinum* and the experiments were discontinued. The protection afforded by the olive oil is probably due to a slowing down of the rate of absorption. It takes some time for the water containing the toxin to separate from the oil. This slowing down of the rate of absorption is sufficient to protect the guinea-pig from small amounts of toxin.

Experiment to determine whether olive oil protects against the action of the toxin of C. botulinum in the stomach of the rabbit when force fed immediately after the toxin and at repeated intervals.—Each rabbit was force fed 2.5 c.c. of a toxic filtrate, Type B. Ten c.c. of olive oil was force fed immediately after the toxin and at repeated intervals. The results obtained (Table 11) indicate that under the conditions of

this experiment olive oil affords a slight protection against the action of the toxin in the stomach of the rabbit. This protection is probably due to a slowing down of the rate of absorption of the toxin. The 2.5 c.c. of toxic filtrate used contained from 2 to 3 M. L. D. of toxin. It is possible that if 1 M. L. D. of toxin were used the protection afforded by the olive oil would be sufficient to save the life of the animal. Since the protection afforded by the olive oil is so slight and is of a physical nature, it is doubtful if it would have had any effect on the course of the disease if the treatment had been begun several hours after the toxin was administered.

TABLE 11.—EFFECT OF OLIVE OIL ON THE ACTION OF TOXIN OF *C. BOTULINUM* IN STOMACH OF RABBIT WHEN FORCE FED IMMEDIATELY AFTER TOXIN AND AT REPEATED INTERVALS

Rabbit	Weight, Gm.	Amount Toxin	Amount Oil	Hours Feeding Oil After Toxin	Results
1	2,300	2.5 c.c.			Dead, 15 hours
2	1,725	2.5 c.c.			Dead, 15 hours
3	1,260	2.5 c.c.	10 c.c.	0, 2, 18, 25	Dead, 39 hours
4	1,860	2.5 c.c.	10 c.c.	0, 2, 18, 25	Dead, 50 hours

LIQUID PETROLATUM

The experiments made with olive oil were repeated with liquid petrolatum as the neutralizing agent. The results obtained are similar to those obtained with olive oil. In experiments made by us the liquid petrolatum seemed to give less protection than the olive oil against the action of the toxin in subcutaneous injections and more protection than the olive oil in the stomach of the rabbit. It is assumed that the protection afforded by the liquid petrolatum is of the same nature as the protection afforded by the olive oil.

Experiment to determine whether liquid petrolatum protects against the action of the toxin of C. botulinum when the two are emulsified, incubated and injected subcutaneously into guinea-pigs.—The liquid petrolatum and a Type A toxic filtrate were mixed in a test tube, emulsified by vigorous shaking and incubated two hours. The mixture was shaken several times during the incubation period. The injections were made subcutaneously into guinea-pigs. The results obtained (Table 12) show that liquid petrolatum protects to a limited extent against the action of the toxin of *C. botulinum*. The guinea-pigs receiving the toxin-liquid petrolatum mixture lived several days longer than the toxin control.

Experiment to determine whether liquid petrolatum protects against the action of the toxin of C. botulinum in the stomach of the rabbit when force fed immediately after the toxin and at repeated intervals.—

This experiment was conducted in the same manner as the similar test with olive oil and the results obtained were approximately the same as with the olive oil. The animal receiving the liquid petrolatum lived eleven hours longer than the animal receiving the olive oil, a slight difference which we believe has no significance. The details of this experiment have no particular significance and are omitted for the sake of brevity. We hoped that a mineral oil which is unaffected by the action of the gastric juice would be more effective against the action of the toxin than a vegetable oil but such did not prove to be the case.

TABLE 12.—EFFECT OF LIQUID PETROLATUM AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE EMULSIFIED, INCUBATED AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Oil	Results
1	600	3 M. L. D.		Dead, 29 hours
2	550	3 M. L. D.	3 c.c.	Dead, 170 hours
3	575	3 M. L. D.	6 c.c.	Dead, 168 hours

BUTTER

Our experiments with butter indicate that it affords less protection against the action of the toxin of *C. botulinum* than olive oil or liquid petrolatum. This is probably due to some physical difference between the butter and the oils. The water containing the toxin seemed to separate from the butter more rapidly than from the oils. If the protection against the toxin afforded by fats depends upon a slowing down of the rate of absorption then the amount of protection afforded by any fat depends on the rate of separation of the water from the fat.

TABLE 13.—EFFECT OF BUTTER AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE EMULSIFIED, INCUBATED AND INJECTED SUBCUTANEOUSLY INTO GUINEA-PIGS

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Butter	Results
1	450	3 M. L. D.		Dead, 21 hours
2	350	3 M. L. D.	3 c.c.	Dead, 35 hours
3	450	3 M. L. D.	6 c.c.	Dead, 50 hours

Experiment to determine whether butter protects against the action of the toxin of C. botulinum when the two are emulsified, incubated and injected subcutaneously into guinea-pigs.—The butter was melted, emulsified with a type B filtrate and incubated two hours. The emulsion was shaken several times during the incubation period. Injections were made subcutaneously into guinea-pigs. The results obtained (Table 13) indicate that butter has a slight retarding action on the toxin of *C. botulinum*. We believe this action is due to a slowing down

of the rate of absorption and that butter does not protect against the toxin as well as olive oil because the emulsion breaks down more rapidly.

MILK, EGG WHITE, EGG YOLK, SUGAR, BRAIN, GELATIN

Kempner and Schepilewsky³⁰ state that the brain tissue of a guinea-pig will neutralize from 3 to 4 M. L. D. of toxin if the brain tissue and toxin are mixed in a test tube and that some protection is afforded by the brain tissue if injected separately from the toxin. Our experiments indicate that the brain tissue of the sheep has no special affinity for the toxin of *C. botulinum* and in "in vitro" injections affords little or no protection.

Experiment to determine whether milk, egg white, egg yolk, sugar, brain tissue and gelatin afford as great protection against the action of the toxin of C. botulinum as olive oil.—The brain tissue was ground up and squeezed through cheese cloth. A type B toxin filtrate was used. All mixtures were shaken thoroughly, incubated 1 hour and injected subcutaneously into guinea-pigs. The results obtained (Table 14) indicate that in subcutaneous injections the protection afforded by these food substances is not equal to that of olive oil.

TABLE 14.—RESULT OF AN EXPERIMENT TO DETERMINE WHETHER MILK, EGG WHITE, EGG YOLK, SUGAR, BRAIN TISSUE AND GELATIN AFFORD AS GREAT PROTECTION AS OLIVE OIL AGAINST ACTION OF TOXIN OF *C. BOTULINUM*

Guinea-Pig	Amount Toxin	Amount Neutralizing Agent	Results
1	3 M. L. D.		Dead in 20 hours
2	3 M. L. D.	3 c.c. olive oil.....	Lived
3	4 M. L. D.	3 c.c. olive oil.....	Dead in 70 hours
4	2 M. L. D.	4 c.c. milk.....	Dead in 20 hours
5	2 M. L. D.	1 c.c. 25% solution glucose.....	Dead in 42 hours
6	2 M. L. D.	3 c.c. egg white.....	Dead in 40 hours
7	2 M. L. D.	3 c.c. egg yolk.....	Dead in 40 hours
8	2 M. L. D.	3 gm. sheep brain.....	Dead in 36 hours
9	3 M. L. D.	3 c.c. 20% gelatin in water.....	Dead in 35 hours

VINEGAR

A number of outbreaks of botulism have followed the eating of salads containing the poisonous food. We considered it advisable to determine whether any of the ingredients of ordinary salad dressing would affect the action of the toxin. The three common ingredients of salad dressings are olive oil, egg and vinegar. We have already shown that olive oil affords some protection against the action of the toxin of *C. botulinum* and that egg affords little or no protection. We record here our experiments with vinegar.

Experiment to determine whether vinegar affords protection against the action of the toxin of C. botulinum when the two are mixed in a test tube, incubated two hours and injected subcutaneously into guinea-

30. Kempner and Schepilewsky: Ueber antitoxische Substanzen gegenüber dem Botulismusgift, Ztschr. f. Hyg. **27**:213, 1898.

pigs.—A Type B toxin filtrate and apple vinegar were used. The toxin and vinegar were mixed in a test tube and incubated two hours and injected subcutaneously in guinea-pigs. The results obtained (Table 15) show that 3 c.c. of the apple vinegar used protected against the action of a 4 M. L. D. of toxin.

It was found that the vinegar caused considerable necrosis, and the possibility of this accounting for the protection against the toxin was considered. It was thought that the necrosis might slow down the rate of absorption and the necrotic cells absorb some of the toxin.

TABLE 15.—EFFECT OF VINEGAR AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE MIXED, INCUBATED TWO HOURS AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Vinegar	Result
1	415	4 M. L. D.		Dead in 15 hours
2	400		3 c.c.	Lived
3	405	4 M. L. D.	3 c.c.	Dead in 84 hours
4	400	4 M. L. D.	3 c.c.	Lived

TABLE 16.—RESULTS OF AN EXPERIMENT TO DETERMINE WHETHER THE PROTECTION AFFORDED BY VINEGAR AGAINST ACTION OF TOXIN OF *C. BOTULINUM* IS DUE TO NECROSIS OF TISSUES OR TO ACETIC ACID PRESENT IN VINEGAR

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Vinegar	Remarks	Results
1	415	4 M. L. D.			Dead, 15 hours
2	430	4 M. L. D.	3 c.c.	Neutralized with sodium hydroxid just before injecting.....	Lived
3	415	4 M. L. D.	3 c.c.	Neutralized before adding to toxin	Lived
4	275	8 M. L. D.	1 c.c.	Neutralized before adding to toxin	Dead, 27 hours
5	280	20 M. L. D.	1 c.c.	Neutralized before adding to toxin	Dead, 15 hours
6	275	200 M. L. D.	1 c.c.	Neutralized before adding to toxin	Dead, 15 hours

Experiment to determine whether the protection afforded by vinegar against the action of the toxin of C. botulinum is due to a neutralization of the toxin or to a necrosis of the tissues or to the acetic acid present in the vinegar.—Vinegar neutralized with sodium hydroxid causes little or no necrosis. The toxin filtrate and vinegar were mixed, incubated two hours, neutralized with normal sodium hydroxid and injected subcutaneously into guinea-pigs. To determine whether neutralized vinegar protects against the action of the toxin, vinegar was neutralized with normal sodium hydroxid, mixed with the toxin, incubated and injected subcutaneously into guinea-pigs. The results obtained (Table 16) indicate that the protection afforded by the vinegar is not due to the necrosis of the tissues. They also indicate that the action on the toxin is not due to the acid in the vinegar since the neutralized vinegar also protects against the action of the toxin.

Experiment to determine whether acetic acid, sodium acetate and sodium hydroxid will protect against the action of the toxin of C.

botulinum when mixed with the toxin incubated and injected subcutaneously into guinea-pigs.—A Type B toxin filtrate was used. The amount of normal sodium hydroxid used was the same as that used to neutralize the 3 c.c. of vinegar. The amount of acetic acid used was the amount required to neutralize the sodium hydroxid. Controls with acetic acid and normal sodium hydroxid were not made. The mixtures were incubated two hours before being injected subcutaneously into guinea-pigs. The results obtained (Table 17) show that acetic acid and sodium acetate do not neutralize the toxin of *C. botulinum*. The guinea-pig receiving the sodium hydroxid-toxin mixture neutralized just before injecting lived nine days. The animal receiving sodium hydroxid toxin mixture unneutralized died in fourteen days. The death of this animal may have been due to the alkali rather than to the toxin, as the whole lower part of the body was greatly swollen. The animal receiving the sodium hydroxid neutralized with acetic acid before the toxin was added died. The protection afforded by the sodium hydroxid apparently is not due to a slowing down of the rate of absorption of the toxin as there was no evidence of necrosis in the animal living nine days. The results obtained with the sodium hydroxid suggest that it neutralizes the action of the toxin to some extent.

TABLE 17.—EFFECT OF ACETIC ACID, SODIUM ACETATE AND SODIUM HYDROXID AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN MIXED WITH TOXIN, INCUBATED AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Neutral Agent and Remarks	Results
1	275	4 M. L. D.	1 c.c. salt solution.....	Dead, 15 hours
2	275	4 M. L. D.	2.4 c.c. normal acetic acid. Neutralized with normal sodium hydroxid just before injecting.....	Dead, 15 hours
3	285	4 M. L. D.	2.4 c.c. normal acetic acid. Neutralized with normal sodium hydroxid before adding to toxin.....	Dead, 36 hours
4	290	4 M. L. D.	2.4 c.c. normal sodium hydroxid.....	Dead, 36 hours
5	300	4 M. L. D.	2.4 c.c. normal sodium hydroxid neutralized with normal acetic acid just before injecting.....	Dead, 14 days
6	400	4 M. L. D.	0.04 gm. sodium acetate.....	Dead, 9 days
7	310	4 M. L. D.	0.04 gm. sodium acetate.....	Dead, 26 hours
8	325	4 M. L. D.	0.04 gm. sodium acetate.....	Lived

These experiments have not determined the active agent in the protection afforded by vinegar or vinegar neutralized with normal sodium hydroxid. We apparently have eliminated acetic acid as a factor in the first case and sodium acetate as a factor in the second case. Since sodium hydroxid reduces the virulence of the toxin, we have not eliminated the possibility of the protection afforded by the vinegar being due to necrosis of the tissues and the protection afforded by the neutralized vinegar being due to some action of the sodium hydroxid. As we have shown that sodium hydroxid neutralized with acetic acid does not reduce the virulence of the toxin we believe that the protection afforded by the vinegar is not due entirely to necrosis but to some factor

not determined by us. A qualitative test for alcohol was positive. Our experiments were discontinued at this point. Armstrong, Story and Scott state that alcohol neutralizes the toxin of *C. botulinum*. The small amount of alcohol in the vinegar may account for its neutralizing action on the toxin. Further experiments with vinegar should be made. In this connection it is interesting to note that Dickson,⁸ quoting Schreiber and Hirschfeldt, reports two outbreaks of food poisoning following the eating of herring preserved in vinegar. Also, some of the outbreaks of botulism in this country followed the eating of salads containing vinegar and olive oil.

TABLE 18.—EFFECT OF VINEGAR AGAINST ACTION OF TOXIN OF *C. BOTULINUM* IN STOMACH OF RABBIT WHEN FORCE FED IMMEDIATELY AFTER TOXIN AND AT REPEATED INTERVALS

Rabbit	Weight, Gm.	Amount Toxin	Amount Vinegar	Hours After Feeding Toxin	Results
1	2,300	2.5 c.c.			Dead, 15 hours
2	1,725	2.5 c.c.			Dead, 15 hours
3	1,260	2.5 c.c.	10 c.c. water	0, 2, 18, 25	Dead, 39 hours
4	1,465	2.5 c.c.	5 c.c. vinegar	0, 2, 18	Lived

Experiment to determine whether vinegar protects against the action of the toxin of C. botulinum in the stomach of the rabbit when force fed immediately after the toxin and at repeated intervals.—A Type B toxin filtrate was used. The amount given was approximately 2 M. L. D. The rabbits were fed 5 c.c. vinegar at each treatment, receiving a total amount of 15 c.c. The results obtained (Table 18) indicate that vinegar affords some protection against the action of the toxin in the stomach of the rabbit.

GRAM'S IODIN SOLUTION

Experiment to determine whether Gram's solution protects against the action of the toxin of C. botulinum when the two are mixed, incubated and injected subcutaneously into guinea-pigs.—A Type B toxin filtrate was used. The Gram solution was mixed with the toxin, incubated two hours and injected subcutaneously into guinea-pigs. The results obtained (Table 19) indicate that this solution protects against the action of the toxin of *C. botulinum*. A guinea-pig was saved from the effects of 8 M. L. D. of toxin by 0.1 c.c. of Gram's solution.

Experiment to determine whether Gram's solution will protect against the action of the toxin of C. botulinum when injected separately from the toxin.—A Type B toxin was used. Repeated injections of Gram's solution were made on the opposite side of the body of the guinea-pigs from the toxin. The injections were begun before or at the same time the toxin was injected. To prevent the iodine causing

necrosis of the tissues, 70 mg. of corn starch were added to each cubic centimeter of the Gram solution. The iodine injections were repeated at three hour intervals. The results obtained (Table 20) indicate that Gram's solution when injected separately from the toxin offers no protection.

TABLE 19.—EFFECT OF GRAM'S SOLUTION AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE MIXED, INCUBATED AND INJECTED SUBCUTANEOUSLY INTO GUINEA-PIGS

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Gram's Solution	Results
1	420	4 M. L. D.		Dead, 28 hours
2	345	8 M. L. D.		Dead, 15 hours
3	275	4 M. L. D.		Dead, 15 hours
4	450	4 M. L. D.	1.5 c.c.	Lived
5	260	4 M. L. D.	0.8 c.c.	Lived
6	275	4 M. L. D.	0.6 c.c.	Lived
7	270	4 M. L. D.	0.4 c.c.	Lived
8	260	4 M. L. D.	0.1 c.c.	Lived
9	345	8 M. L. D.	0.1 c.c.	Lived
10	335	20 M. L. D.	0.1 c.c.	Dead in 40 hours
11	320	200 M. L. D.	0.1 c.c.	Dead in 15 hours

TABLE 20.—EFFECT OF GRAM'S SOLUTION AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN INJECTED SEPARATELY FROM THE TOXIN

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Gram's Solution	Hours Injected After Toxin	Results
1	315	2 M. L. D.			Dead, 35 hours
2	315	2 M. L. D.	4 c.c.	0, 3, 5, 9	Dead, 29 hours
3	280	2 M. L. D.	4 c.c.	3, 0, 3, 5, 9	Dead, 31 hours

TABLE 21.—RESULT OF EXPERIMENT TO DETERMINE WHETHER THE PROTECTION AFFORDED BY GRAM'S SOLUTION AGAINST ACTION OF TOXIN OF *C. BOTULINUM* IS DUE TO THE NECROSIS OF THE TISSUE, TO THE POTASSIUM IODID OR TO THE IODIN

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Neutralizing Agent and Remarks	Results
1	335	4 M. L. D.		Dead, 15 hours
2	450	4 M. L. D.	1.5 c.c. Gram's solution.....	Lived
3	465	4 M. L. D.	1.5 c.c. Gram's solution, starch added just before injecting.....	Lived
4	460	4 M. L. D.	1.5 c.c. Gram's solution, starch added before the toxin.....	Lived
5	450	4 M. L. D.	3 c.c. saturated solution of iodine.....	Lived
6	420	4 M. L. D.	1.5 c.c. 2% potassium iodid.....	Dead, 40 hours
8	435	4 M. L. D.	105 mg. corn starch in salt solution.....	Dead, 28 hours
7	210	4 M. L. D.	4.5 c.c. 2% potassium iodid.....	Dead, 27 hours

Experiment to determine whether the protection afforded by Gram's solution against the action of the toxin of C. botulinum is due to the necrosis of the tissues, to the potassium iodid or to the iodine.—A Type B toxin filtrate, a 2 per cent. solution of potassium iodid and a saturated aqueous solution of iodine were used. Seventy mg. of corn starch were added to each cubic centimeter of the iodine solution to prevent necrosis of the tissues. The toxin and solutions were mixed, incubated two hours

and injected subcutaneously into guinea-pigs. The animals receiving the Gram solution, the Gram iodine starch mixture and the saturated solution of iodine lived. The animal receiving the potassium iodide died about the same time as the controls. The results obtained (Table 21) show that the protection afforded by Gram's solution is not due entirely to necrosis of the tissues or to the potassium iodide present, but to the iodine.

Experiment to determine whether Gram's solution neutralized with corn starch is just as effective as unneutralized Gram's solution in protecting against the action of the toxin of C. botulinum.—A Type B toxin filtrate was used. To neutralize the action of the Gram's solution on the tissues, 70 mg. of corn starch was added to each cubic centimeter. The toxin was mixed with the solutions, incubated two hours and injected subcutaneously into guinea-pigs. The results obtained (Table 22) indicate that the addition of starch to the Gram solution slightly reduces its protective action against the toxin. The increased protection afforded by the unneutralized Gram's solution may be due to its action on the tissues.

TABLE 22.—RESULTS OF EXPERIMENT TO DETERMINE WHETHER GRAM'S SOLUTION NEUTRALIZED WITH CORN STARCH IS JUST AS EFFECTIVE AS UNNEUTRALIZED GRAM'S SOLUTION IN PROTECTING AGAINST ACTION OF TOXIN OF C. BOTULINUM

Guinea-Pig	Weight	Amount Toxin	Amount Neutralizing Agent	Result
1	420	4 M. L. D.		Dead, 28 hours
2	275	4 M. L. D.		Dead, 15 hours
3	260	4 M. L. D.	0.1 c.c. Gram's solution.....	Lived
4	345	8 M. L. D.	0.1 c.c. Gram's solution.....	Lived
5	335	20 M. L. D.	0.1 c.c. Gram's solution.....	Dead, 40 hours
6	320	200 M. L. D.	0.1 c.c. Gram's solution.....	Dead, 15 hours
7	465	4 M. L. D.	0.4 c.c. neutralizing Gram's solution	Lived
8	380	8 M. L. D.	0.4 c.c. neutralizing Gram's solution	Dead, 110 hours
9	360	20 M. L. D.	0.4 c.c. neutralizing Gram's solution	Dead, 18 hours
10	385	200 M. L. D.	0.4 c.c. neutralizing Gram's solution	Dead, 15 hours

TABLE 23.—EFFECT OF GRAM'S SOLUTION AGAINST ACTION OF TOXIN OF C. BOTULINUM IN STOMACH OF RABBIT WHEN FORCE FED IMMEDIATELY AFTER TOXIC CULTURE AND AT REPEATED INTERVALS

Rabbit	Amount Toxic Culture	Amount Gram's Solution	Hours After Toxin	Results
1	6 c.c.			Dead, 17 hours
2	6 c.c.			Dead, 30 hours
3	6 c.c.	4 c.c.	0	Dead, 3 hours
4	6 c.c.	4 c.c.	0, 2, 4	Lived

Experiment to determine whether Gram's solution will protect against the action of the toxin of C. botulinum in the stomach of the rabbit when force fed immediately after a toxic culture and at repeated intervals.—Each rabbit received 6 c.c. of a toxic meat culture of C. botulinum Type B. Four c.c. of Gram's solution was used at each

treatment. This is an extreme dose and will kill some of the rabbits before the toxin has time to act. We did not test the effect of a Gram iodine-starch solution. The Gram solution is a strong disinfectant and should destroy the organisms reached as well as the toxin. In our short series of experiments one of the rabbits receiving the Gram solution after the toxin lived, indicating that the iodine has some neutralizing action on the toxin in the stomach of a rabbit (Table 23).

LIQUID SOAP

We have already shown that sodium hydroxid has a neutralizing action on the toxin of *C. botulinum*. Since soap contains an alkali and may be used in the preparation of enemas, we undertook to determine whether liquid soap would protect against the action of the toxin of *C. botulinum*. The soap used did not change the color of either litmus paper or phenolphthalein paper.

TABLE 24.—EFFECT OF LIQUID SOAP AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE MIXED, INCUBATED AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Soap	Remarks	Results
1	275	4 M. L. D.			Dead, 15 hours
2	305	4 M. L. D.	1 c.c.	3 c.c. salt solution added just before injecting...	Lived
3	315	8 M. L. D.	1 c.c.	3 c.c. salt solution added just before injecting...	Lived
4	330	20 M. L. D.	1 c.c.	3 c.c. salt solution added just before injecting...	Lived
5	325	200 M. L. D.	1 c.c.	3 c.c. salt solution added just before injecting...	Lived

Experiment to determine whether liquid soap will protect against the action of the toxin of C. botulinum when the two are mixed, incubated and injected subcutaneously into guinea-pigs.—A Type B. toxin filtrate was used. Subcutaneous injection of the soap caused considerable necrosis and occasionally death. The addition of salt solution was found to prevent this, and in these experiments 3 c.c. of physiologic sodium chlorid solution were added to each cubic centimeter of soap used. The salt solution was added to the toxin-soap mixture after the incubation period. The toxin-soap mixtures were incubated two hours, the salt solution added, and then injected subcutaneously into guinea-pigs. The results obtained (Table 24) show that 1 c.c. of the liquid soap used protected a guinea-pig from at least 20 M. L. D. of toxin. No attempt was made to determine the active agent in the neutralization of the toxin, but we believe that the alkali in the soap neutralized the toxin. We found that glycerin has no effect on the toxin.

Experiments to determine the action of soap on the toxin in the stomach of rabbits was begun but were not carried far enough to war-

rant description. In our preliminary experiments in force feeding soap, the animals died in a few hours from the effects of the soap. Experiments should be made to determine whether the soap can be diluted sufficiently not to injure the rabbit fatally and retain its action on the toxin. The action of the soap on the toxin in the intestines should also be demonstrated. Experiments with soap might lead to some treatment of value.

MISCELLANEOUS FEEDING EXPERIMENTS

Drawing any conclusions from the results of a short series of feeding experiments is very unsatisfactory. This is particularly true when feeding small amounts of toxin and basing the conclusions on the time of appearance of the symptoms or the time of death. However, these are conditions that are difficult to avoid. We are dealing with a powerful toxin and we cannot hope for more than a slight protection from any nonspecific treatment. In subcutaneous injections the lethal dose can be accurately determined and remains fairly constant for animals of the same weight. This is not true in feeding experiments. As a result of our experiment to determine the effect of a full rabbit stomach on the action of the toxin, we believe that the amount of food in the rabbit stomach at the time the animal is fed will affect the time of death. Also, if the feeding tube penetrates the mass of food in the stomach and the toxin is deposited in the center of the food mass, absorption will be delayed. Some rabbits readily take food after the toxin is fed, others eat sparingly or not at all. In order to discount these factors, we used two toxin controls and sufficient toxin to insure death in less than forty-eight hours. Presumably any animal living longer than forty-eight hours was protected to some extent by the substance being tested. The amount of toxin used was such that in most cases the toxin controls were dead in fifteen hours. While the animals living longer than forty-eight hours apparently were protected to some extent, it is possible that some of the animals dying between twenty-four and forty-eight hours were also slightly protected.

We believe that with most of the substances used by us the main factor in the protection afforded is a slowing down of the rate of absorption. We have shown in subcutaneous injection that by mixing the toxin with olive oil, the rate of absorption is slowed down to such an extent that guinea-pigs will resist three lethal doses. The rate of absorption of the toxin from the stomach affects the time of the appearance of symptoms and of death. And, in case a small amount of toxin is ingested, any slowing down of the rate of absorption may prevent death.

When it became evident that our investigations must be terminated, we carried out a number of feeding experiments. We had already shown that some of the substances used had no effect on the toxin when mixed with it and injected subcutaneously. It was thought, however, that they might delay the appearance of the symptoms when force fed into the stomach immediately after the toxin. The results obtained would, at least, have some bearing on our belief that the amount and kind of food taken with the toxin materially affects the time of appearance and severity of the symptoms. Also, these non-neutralizing substances might have more effect on the toxin after coming in contact with and being altered by the gastric juice. Other of these substances had been shown to protect against the action of the toxin in subcutaneous injections. It was thought that these might not have any effect on the toxin in the stomach as a result of being altered by contact with the gastric juice before coming in contact with the toxin.

Experiment to determine the effect of milk, turpentine, alcohol, a proprietary nerve tonic and water on the toxin in the stomach of the rabbit when force fed immediately after the toxin and at repeated intervals.—About 2 M. L. D. of Type B toxin was used; this required 2.5 c.c. of the filtrate. We have shown that milk does not neutralize or protect against the action of the toxin in subcutaneous injections. Armstrong, Story and Scott have shown that alcohol will protect against the action of the toxin when the two are mixed and injected subcutaneously. We used a 30 per cent. solution of alcohol. While testing the protective properties of miscellaneous substances it was suggested that the effects of a proprietary nerve tonic be determined. According to the label this tonic contained 14 per cent. alcohol, and each fluid ounce contained 8 grains sodium glycerophosphate, 4 grains calcium glycerophosphate, 4 grains potassium glycerophosphate, $\frac{1}{60}$ grain strychnin glycerophosphate, $\frac{1}{16}$ grain lecithin and $\frac{1}{30}$ grain avenin. The tonic was used without diluting. These substances were force fed immediately after the toxin and at repeated intervals. The results obtained (Table 25) indicate that either numerous substances have a slight effect on the action of the toxin in the stomach of the rabbit, or that none of the substances used had any effect, and the results obtained simply represent individual variation in resistance to the toxin. Rabbits do show considerable variation in resistance to force fed toxin. We believe, however, that our results indicate something more than individual variation or error in technic. In experiments of this kind in which we are endeavoring to determine or measure slight effects and in which all the factors cannot be controlled, long series of experiments should be made. We have not had an opportunity to do this.

TABLE 25.—RESULTS OF EXPERIMENTS TO DETERMINE EFFECT OF MILK, TURPENTINE, ALCOHOL, A PROPRIETARY NERVE TONIC AND WATER ON THE TOXIN OF *C. BOTULINUM* IN THE STOMACH OF RABBIT WHEN FORCE FED IMMEDIATELY AFTER TOXIN AND AT REPEATED INTERVALS

Rabbit	Weight, Gm.	Amount Toxin	Amount Neutralizing Agent	Injections Hours After Toxin	Results
1	2,300	2.5 c.c.			Dead, 15 hours
2	1,725	2.5 c.c.			Dead, 15 hours
3	1,675	2.5 c.c.	10 c.c. milk.....	0, 2, 18, 25	Dead, 87 hours
4	1,575	2.5 c.c.	5 c.c. tonic.....	0, 2, 18, 25	Lived
5	1,800	2.5 c.c.	4 c.c. turpentine..	0, 5	Dead, 80 hours
6	2,000	2.5 c.c.	4 c.c. 30% alcohol	0, 5	Dead, 73 hours
7	1,260	2.5 c.c.	15 c.c. water.....	0, 2, 18, 25	Dead, 39 hours

EPINEPHRIN

Marie³¹ states that epinephrin neutralizes the soluble toxins of tetanus and diphtheria. He shows that epinephrin will protect against lethal doses of the toxins both in "in vitro" and in "in vivo" experiments. Our experiments indicate that epinephrin chlorid affords some protection against lethal doses of the toxin of *C. botulinum* when the two are mixed and injected subcutaneously.

Experiment to determine whether epinephrin chlorid will protect against the action of the toxin of C. botulinum when the two are mixed, incubated and injected subcutaneously into guinea-pigs.—A Type B toxin filtrate and the ordinary commercial epinephrin chlorid solution, 1:1,000, were used. The toxin and epinephrin solution were mixed, incubated two hours and injected subcutaneously in guinea-pigs. The results obtained (Table 26) indicate that epinephrin chlorid solution has some neutralizing action on the toxin of *C. botulinum*. Experiments should be made to determine whether the action of epinephrin on the body influences the course of the disease.

TABLE 26.—EFFECT OF EPINEPHRIN CHLORID AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE MIXED, INCUBATED AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Weight, Gm.	Amount Toxin	Amount Epinephrin	Results
1	175	3 M. L. D.		Dead, 18 hours
2	200		0.1 c.c.	Lived
3	200	3 M. L. D.	0.1 c.c.	Dead, 42 hours
4	210	3 M. L. D.	0.2 c.c.	Dead, 60 hours

POTASSIUM PERMANGANATE

Experiment to determine whether potassium permanganate will protect against the action of the toxin of C. botulinum when the two are mixed, incubated and injected subcutaneously in guinea-pigs.—A

33. Marie: Du mode d'action de l'adrenaline vis a vis des toxines solubles, *Compt. rend. Soc. de biol.* **82**: 1919; Du mode d'action de l'adrenaline sur les toxines bacteriennes, *Ann. de l'Inst. Pasteur* **33**:645, 1919.

Type A toxin filtrate and a 1 per cent. water solution of potassium permanganate were used. The toxin and potassium permanganate solution were mixed, incubated two hours and injected subcutaneously into guinea-pigs. The results obtained (Table 27) indicate that 1 c.c. of a 1 per cent. solution of potassium permanganate will protect a guinea-pig from at least 6 M. L. D. of toxin. The potassium permanganate causes considerable necrosis of the tissues. No attempt was made to determine if the necrosis is a factor in the protection afforded by the potassium permanganate.

TABLE 27.—EFFECT OF POTASSIUM PERMANGANATE AGAINST ACTION OF TOXIN OF *C. BOTULINUM* WHEN THE TWO ARE MIXED, INCUBATED AND INJECTED SUBCUTANEOUSLY

Guinea-Pig	Amount Toxin	Amount Potassium Permanganate	Result
1	2 M. L. D.		Dead, 36 hours
2	2 M. L. D.	1 c.c.	Dead, 15 days
3	3 M. L. D.	1 c.c.	Lived
4	4 M. L. D.	1 c.c.	Lived
5	6 M. L. D.	1 c.c.	Lived

CONCLUSIONS

1. Spoiled foods containing gas may appear to be boiling for several minutes before the true boiling point is reached. We recommend that all suspected food be subjected to vigorous boiling for at least thirty minutes before being tasted.

2. Spoiled canned foods giving the appearance of boiling for seven minutes and subjected to actual boiling for four minutes are not safe to eat. Spoiled canned foods exposed to a temperature of 80 C. for one hour may appear to be boiling part of the time and not be safe to eat.

3. We may expect to have outbreaks of botulism following the eating of insufficiently cooked spoiled foods.

4. The heat resistance of the disease producing power of different kinds of spoiled canned foods containing *C. botulinum* and its toxin has not been determined and probably will be found to vary.

5. There have been no recorded outbreaks of botulism in this country without a history of preserved foods having been eaten.

6. There is no evidence that infection in man ever follows the ingestion of toxin free organisms of *C. botulinum*.

7. There is no evidence that infection in man ever follows the ingestion of the toxin and organisms of *C. botulinum*.

8. Botulism does not result from the ingestion of small numbers of toxin free spores.

9. The disease producing power of an old can of spoiled beans containing *C. botulinum* and its toxin can be destroyed by an exposure to temperatures that rarely if ever destroy the spores of *C. botulinum*.

10. Rabbits can be saved from the effects of feeding 2 M. L. D. of a toxic culture of *C. botulinum* by the intravenous injection of the homologous antitoxin as soon as the symptoms appear. Such rabbits cannot be saved if the antitoxin treatment is delayed until the symptoms are well advanced.

11. In outbreaks of botulism, as soon as the first case develops, a polyvalent immune serum should be injected intravenously in all those who have partaken of the suspected meal. This treatment should materially decrease the death rate.

12. The contents of a full normal rabbit stomach does not neutralize the toxin of *C. botulinum* but it apparently reduces the action of the toxin to some extent by slowing down or preventing its absorption.

13. Fats and oils do not neutralize the toxin of *C. botulinum*.

14. Fats and oils will protect against the action of the toxin of *C. botulinum* when the toxin and fat or oil are emulsified and injected subcutaneously into guinea-pigs. This protection is very limited and varies with the degree of the emulsion and the type of fat. The protection is due to a slowing down of the rate of absorption of the toxin.

15. Olive oil will delay the action of the toxin in the stomach of a rabbit when force fed immediately after the toxin and at repeated intervals. It apparently affords no greater protection than milk.

16. Certain food substances, such as glucose, brain tissue, milk, eggs and gelatin do not neutralize the toxin of *C. botulinum* and do not protect as effectively as olive oil against the action of the toxin in subcutaneous injections.

17. Apple vinegar, giving a positive test for alcohol, protects against the action of the toxin of *C. botulinum* when the two are mixed, incubated two hours and injected subcutaneously into guinea-pigs.

18. The protection afforded by the vinegar is not due to necrosis of the tissues, since vinegar neutralized with sodium hydroxid does not cause necrosis, and still protects against the action of the toxin. The protection afforded by the vinegar may be due to the alcohol present. Acetic acid and sodium acetate do not neutralize the toxin of *C. botulinum*.

19. Vinegar when force fed immediately after a toxin filtrate and at repeated intervals afforded some protection against the action of the toxin in the stomach of the rabbit.

20. Sodium hydroxid, potassium permanganate and liquid soap have a neutralizing action on the toxin of *C. botulinum*.

21. Epinephrin chlorid has a slight neutralizing action on the toxin of *C. botulinum*.

22. Iodin and iodine to which sufficient starch has been added to prevent necrosis of the tissues has a strong neutralizing action on the toxin of *C. botulinum*. Potassium iodide does not neutralize the toxin of *C. botulinum*. In "in vivo" experiments, iodine injected on the opposite side of the body from the toxin afforded no protection. Gram's solution affords some protection against the action of the toxin in the stomach of the rabbit when force fed immediately after the toxin.

SUMMARY

The treatment of botulism is in the experimental stage, and based mainly on insufficient or inexact knowledge. In experimental work with laboratory animals and in the treatment of human cases of botulism there has not been gained sufficient knowledge to develop a scientific treatment. This can be explained partly on the grounds that it is only within the past few years that the attention of the investigators has been drawn to the subject of botulism and partly to the fact that we are dealing with a powerful and rapidly acting toxin. However, we have reasons to believe that when further experimental work has been done, and physicians become better acquainted with the disease, a treatment will be developed that will materially lessen the death rate.

Specific Treatment.—The treatment of botulism may be classed as specific and general. By specific treatment we mean the use of an immune serum which neutralizes the specific toxin of *C. botulinum*. Since there are at least two distinct toxins, and there is no rapid means of determining the type, it is necessary to use a polyvalent antitoxin or a Type A and a Type B antitoxin. And since there is a possibility that *C. botulinum* occasionally produces its toxin in the body, the immune serum should be bacteriolytic or bactericidal as well as antitoxic. The serum should be injected intravenously.

The immune serum to be beneficial must be used as soon as possible after the diagnosis is made. In all cases of doubtful diagnosis, all of those having partaken of the suspected meal should receive immediate antitoxin treatment. The antitoxin can only neutralize the toxin and prevent further injury. It is of no value to the already damaged nervous system. The evidence we have indicates that treatment begun after the symptoms are well advanced will not, in most cases, alter the course of the disease. But we have sufficient experimental evidence to believe that some of those receiving lethal amounts of toxin can be saved by the use of antitoxin if the treatment is begun at about or before the time the symptoms appear.

General Treatment.—The general treatment of botulism consists of treatment of the digestive tract and stimulation of the nervous system.

Botulinus toxin is a true toxin and differs from the toxins of diphtheria and tetanus in that it is, so far as we know, taken into and possibly produced in the digestive tract and is not altered by the gastric juice. This gives us an opportunity to develop a treatment directed against the toxin in the digestive tract as well as in the blood stream.

Treatment of the digestive tract in cases of botulism has been designed to prevent the further absorption of the toxin by its removal, stimulation of gastric secretions, the use of demulcents and oils. A method of neutralizing the toxin in the digestive tract should also be developed.

Intestinal Tract: Treatment of the intestinal tract in cases of botulism cannot be placed on a scientific basis until we know the effect of the intestinal contents on the toxin. Graham has shown that the droppings of chickens suffering from botulism and also droppings found in forage causing botulism when ground up and fed to horses caused death. He apparently assumes that the toxin passed through the intestinal tract of the chickens and that the horses died as the result of the ingestion of this toxin rather than from the production of toxin in the digestive tract following the ingestion of toxin-free organisms. Forssman has shown that the injection of 2,000 lethal doses of toxin in the rabbit intestine does not cause death. He believed that the intestinal contents of the rabbit precipitated the toxin. Until we know whether or not the toxin in the intestinal tract is harmful we must proceed upon the assumption that it is and take steps for its removal, neutralization and the prevention of absorption.

We recommend the use of high enemas that will neutralize the toxin, prevent its absorption and destroy the organisms. We have shown that some of the common ingredients of enemas should be beneficial. Liquid soap neutralizes the toxin, olive oil prevents its absorption and turpentine may have some beneficial action. Iodin and potassium permanganate destroy both the toxin and organisms and might be used to advantage. The effect of soap on the organism is unknown and should be determined. If it can be shown that the toxin in the intestinal tract is harmless and is not produced there, the use of enemas can be restricted to feeding and the control of constipation.

Stomach: In those patients in which the symptoms do not appear for several days after the ingestion of the toxic food, we may reasonably assume that there is very little, if any, of the ingested toxin remaining in the digestive tract. But since there is a possibility of infection and production of toxin in the digestive tract, it is necessary to proceed on the assumption that toxin is always present in the stomach of any one suffering from botulism. When the symptoms appear in a

few hours after the ingestion of the poisonous food we should expect to find toxin in the stomach. In these cases paralysis of the stomach apparently sets in early. Case reports show that toxin has been found in the stomach several days after the ingestion of the toxic food.

Treatment of the stomach should have in mind the removal of the toxic contents by stimulation of movement or the stomach pump, the prevention of absorption by the use of demulcents and oils or stimulation of secretions, the neutralization of the toxin and the destruction of the organisms. The removal of the stomach contents is attended with some danger as it may induce pneumonia. The death rate among those vomiting is 75 per cent.; among those not vomiting, 70 per cent. The average time of death of those vomiting is 3.7 days and of those not vomiting 5 days after the ingestion of the toxic food. This indicates that those ingesting the most toxin tend to vomit or the act of vomiting induces a terminal pneumonia.

In our experimental work we have shown that the food taken in with the toxin delays the appearance of the symptoms. This is probably due to a slowing down of the rate of absorption of the toxin. Our work has also shown that a slowing down of the rate of absorption enables a guinea-pig to resist 3 lethal doses of toxin. The use of oils, milk and alcohol slowed down the action of the toxin in the stomach of the rabbit. Iodin, potassium permanganate and liquid soap have a strong neutralizing action on the toxin but it has not been determined whether they can safely be used in the stomach in large enough amounts to be beneficial.

Treatment of the stomach must be begun early, as paralysis of the throat soon sets in and there is great danger of pneumonia. Most of our rabbits dying after the fourth day showed gray hepatization of the lungs. A nonirritating gargle of 2 per cent. argyrol may be used as a precautionary measure.

Throat: Pilocarpin has been used to remove the tenacious mucus usually found in the throat in advanced cases. It "should be used with caution as the patient is unable to cough up fluid from the lungs if pulmonary edema is induced."

Nervous system: Treatment of the nervous system must be general until we know what nerves or nerve centers are affected. Strychnin has been used in most cases.

Supportive measures: The supportive measures in use have been mentioned above.

We cannot hope for a more satisfactory treatment until our knowledge of the subject becomes more exact. With our present knowledge of botulism, the death rate should be reduced when antitoxin becomes more available and the symptoms more readily recognized.

PROGNOSIS

From an examination of the incomplete statistical data we obtain certain facts which have a bearing on the prognosis. The death rate in this country varies from 60 to 70 per cent. The death rate among those first showing symptoms in all outbreaks in this country is 90 per cent., among those last showing symptoms 60 per cent. The death rate of those vomiting is 75 per cent. Vomiting cannot be considered a favorable sign. The death rate of those showing symptoms in twenty-four hours after the ingestion of the toxic food is 84 per cent.; of those showing symptoms for the first time after seventy-two hours it is 55 per cent., and of those alive after the eighth day it is 20 per cent.