MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

No. 8 July 31, 1918

BOTULISM

A Clinical and Experimental Study

By

ERNEST C. DICKSON, M.D.

NEW YORK
The Rockefeller Institute for Medical Research
1918

MONOGRAPH No. 8

BOTULISM

A CLINICAL AND EXPERIMENTAL STUDY

BY

ERNEST C. DICKSON, M.D.

NEW YORK
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
1918

MONOGRAPH OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
NO. 8, July 31, 1918.

BOTULISM.

A Clinical and Experimental Study.

By ERNEST C. DICKSON, M.D.

(*From the Division of Medicine of Stanford University Medical School, San Francisco.*)

PLATES 1 TO 10.

(Received for publication, September 5, 1917.)

INTRODUCTION.

The term *Botulismus* (*botulus*, a sausage) was coined by the physicians of Southern Germany in the beginning of the XIXth century, to apply to a peculiar type of food-poisoning which was caused by the ingestion of spoiled sausages. Synonymous terms which are in use in the literature are allantiasis and *Wurstvergiftung*. When the term was first applied, its use was restricted to cases of sausage-poisoning, but gradually it became known that the symptom complex which is produced by sausage-poisoning may also be produced by poisoning from preserved, usually smoked, meat and fish, and that it may be closely simulated by poisoning from cheese. For many years its application was often inaccurate as there was much confusion concerning the different types of illness which were known to follow the ingestion of spoiled foods; but in 1895 van Ermengem succeeded in isolating a bacterium from ham which had caused the illness of twenty-three persons, in all of whom the symptoms were those of true botulism, and was able to prove that in botulism the poisoning is due to a toxin which is produced when the bacillus is grown upon suitable culture medium. The present day significance of the term botulism is therefore restricted to poisoning by the toxin of *Bacillus botulinus*, and includes poisoning from any foodstuff in which the bacillus has grown and has produced its toxin.

The investigation which is recorded in this report was commenced in December, 1913, at which time twelve persons were poisoned at a banquet in a student's club-house at the Leland Stanford Junior University. The clinical picture and the course of the illness were in all respects identical with those of botulism. The outbreak was of peculiar interest in that the only article of food of which all the patients had partaken was not meat or fish, but was salad prepared from string beans which had been canned at the home of one of the members of the club. A further point of interest was revealed when histologic examination was made of the tissues of the one patient who died as a result of the poisoning. Professor Ophüls, of the Stanford University Med-

ical School, observed a peculiar type of thrombosis in the blood vessels of the meninges and of the brain tissue, a lesion which had not previously been described in victims of poisoning from botulism. Proof that the illness was due to the ingestion of the toxin of *Bacillus botulinus* was lacking, as there were no remnants of the salad which could be examined, and *Bacillus botulinus* was not recovered from the organs of the patient who died.

My experimental work was undertaken to determine whether it is possible that the toxin of *Bacillus botulinus* may be formed in canned string beans in which there is no addition of protein of animal origin, and whether the thrombosis is a lesion which is characteristic of poisoning by the toxin. Subsequently, the investigation was extended to include other vegetables and fruits, and the importance of determining whether the toxin may be formed in foodstuffs which are not of animal origin was emphasized by the fact that there were several more outbreaks of a similar type in various portions of the States of California and Oregon, all of them following the ingestion of home-canned vegetables or fruits.

It has been thought that botulism is exceedingly rare in this country. It is only in the larger Systems of Medicine that the symptomatology is discussed in detail, and there are very few reported cases in the American literature in which the diagnosis has been made. It has been generally understood that the intoxication is one of the group of poisonings from contaminated meat, and the importance of vegetables and fruits as a possible source of poisoning has not been recognized.

Because of the fact that there is no complete review of the history of botulism in the English language, and because of the importance which this type of food-poisoning has assumed in the United States, particularly upon the Pacific Coast, the subject is discussed in much greater detail than would otherwise be justifiable.

HISTORICAL REVIEW.

EUROPEAN LITERATURE.

Incidence and Geographical Distribution.

Botulism has been recognized by German physicians since the latter part of the XVIIIth century. The first recorded case occurred in 1735 (1), and others were described in 1755 (2) and 1789 (3); but the outbreak which seems to have attracted the attention of the medical profession occurred in 1793 (4), in Wildbad in Würtemberg, where thirteen persons became ill and six died after eating "*Schweinsmagen*" or "*Blunzen*," sausage which was packed in the stomach of a hog, and which contained a great deal of blood. The number of cases rapidly increased after this time and became so numerous that in 1802 Jaeger (5) published an official warning from Stuttgart, in which he detailed the symptoms of the condition, drew attention to the danger of eating spoiled sausages, and gave instructions concerning the proper method of preparing and curing sausages.

In spite of this warning the number of cases of sausage-poisoning in Würtemberg steadily increased. In 1815 von Autenrieth (6) published a report of a series of four cases which had been observed by two of his students, Kerner and Steinbach. In 1820 Kerner (7) published a monograph in which he recorded seventy-six cases of sausage-poisoning, of which thirty-seven had been fatal; and in 1822 he published a second monograph (8) in which he recorded eighty-four new cases of which forty-seven were fatal. In 1824 Weiss (9) reported twenty-nine new cases, of which three were fatal, and in 1828 Dann (10) recorded that, between the years 1793 and 1827, there had been 234 cases of sausage-poisoning in Würtemberg, of which 110 were fatal.

The rapid increase in the number of recorded cases was largely due to the efforts of Kerner, who had been instrumental in having laws enacted which required the registration of all cases of sausage-poisoning within the Kingdom of Würtemberg. He was so impressed with the importance of this type of food-poisoning in Würtemberg that he wrote: "Es ist möglich, dass in einem gleich grossen Landstriche der tropischen Länder nicht viel mehr Menschen durch Schlangengift, als wie bei uns durch diess unselige Wurstgift siech und getödtet werden" (7).

The earlier cases were confined to Würtemberg and to the adjoining portions of Baden and Bavaria, but gradually isolated cases were recorded in other portions of Germany. In 1821 Kahleis (11) reported the first case in Anhalt, in Gröbzig, and, later, cases were also described in Dessau. In the interval between 1820 and 1830 several outbreaks were described in Westphalia, and in 1822 and

in 1828 official warnings were published by the authorities at Arnsberg (12). The majority of cases, however, continued to occur in Würtemberg, and in 1852 Schlossberger (13) recorded that between 1800 and 1850 there had been about 400 cases of sausage-poisoning in Würtemberg, of which 150 had been fatal, while in 1862 von Faber (14) reported that between 1832 and 1862 there had been at least 82 cases, of which 19 had died. Subsequent to 1860 the occurrence in Würtemberg has been less frequent, but cases in other portions of Europe have been more frequent. Senckpiehl (15) in 1887 collected 412 cases, 165 fatal, which had occurred during the period between 1789 and 1886, and in 1909 Wosnitza (16) added 59 additional cases, of which only four died. Several outbreaks have been described since 1909. In 1913 Bürger (17) recorded twelve cases of which five died, and Schumacher (18) described six cases of which one died. In 1915 Paulus (19) reported four cases of which two died, and Hoeg (20) recorded one in Denmark which recovered. The most recent report which I have found is that of Schede (21), 1916, who described five cases of which two died.

The collection of cases by Senckpiehl (15), Kaatzer (22), Wosnitza (16), and Paulus (19) includes many which occurred in various portions of Germany and a few from other countries. Cases are recorded which occurred in Würtemberg, Baden, Bavaria, Prussia, Pomerania, Hessen, Westphalia, Saxony, Anhalt, Hanover, Posen, and Silesia. Several reports occur from Russia, Austria, Hungary, and Denmark. One outbreak was described in England in 1860 (23), and one in France in 1875 (24), in which it was stated that the poisonous sausages had been prepared in England. Two other outbreaks were described in France, one in Holland, and two, including the one in which van Ermengem established the etiology of the poisoning, occurred in Belgium.

A review of the medical literature indicates that outbreaks of botulism have been considerably less frequent in Germany during the past 50 years, but in an elaborate discussion of food-poisoning in which the data were obtained from official records, Mayer (25), in 1913, showed that it is still comparatively frequent. The following table is summarized from one in his report:[1]

From	1793–1820,	76 cases,	37 fatal.
"	1820–1822,	98 "	34 "
"	1822–1886,	238 "	94 "
Since	1886, about	800 "	about 200 fatal.

Types of Foodstuffs Infected.

The symptoms of botulism have developed after the ingestion of various kinds of foods. In the report by Jaeger (5) in 1802 only blood sausages, "*Blutwürste*," were mentioned, but in 1815 von Autenrieth (6) reported an outbreak in which liver sausages, "*Leberwürste*," were the cause of the poisoning. Weiss (9) in 1827 described a case which developed after the ingestion of a sausage prepared from

[1] Mayer (25), p. 57.

goat blood and sheep plucks, and in 1830 Horn (26) referred to cases due to "*Schlackwurst,*" prepared from pork to which is added veal and calf blood, and "*Presskopf*" prepared from livers or tongues and meat from the heads of hogs. In practically all the subsequent outbreaks in which sausages have been responsible, the sausages were of the varieties which were recognized so early.

Kerner (7) noted that meat sausages were less frequently infected than blood or liver sausages, and he believed that this was due to the fact that they were not cooked before they were smoked. Schlossberger (13) agreed that meat sausages were rarely affected, but he noted that they were usually packed in small casings, and that, as they were more expensive, and were used chiefly by the wealthier classes, they were more likely to be prepared by skillful butchers under cleanly conditions. All the authors agreed that sausages which were packed in large casings, such as the stomach and large intestine, were more apt to be poisonous than those which were packed in small casings, and as early as 1824 Kerner (27) advised against the use of large casings.

Outbreaks due to the eating of smoked ham were described as early as 1822 (15), the one which was responsible for the official warning in Arnsberg being the first recorded. Hauff (28) in 1829 described a case in which the patient had eaten smoked pork, and numerous other authors have recorded cases in which ham was responsible for the poisoning. Geiseler (29) in 1824, Thorer (30) in 1834, Ulrich (31) in 1882, Roth (32) in 1883, Groenouw (33) in 1890, van Ermengem (34) in 1897 and in 1906 (87), Römer (35) in 1900, Blattmann (36) in 1909, Bürger (17) in 1913, Schumacher (18) in 1913, and Schede (21) in 1916, have all placed the responsibility for the poisoning of their patients upon smoked and pickled ham, and Roth also recorded one series of cases in which smoked bacon was responsible. In Paulus' series (19) the poisoning was traced to poorly salted pork.

Autenrieth in 1833 and 1835 (37) referred to the similarity of the symptoms of the sausage-poisoning of Würtemberg and those of the smoked fish-poisoning of certain parts of Russia. In 1850 Jaechnichen (38) described a series of cases of fish-poisoning in Russia and noted the close resemblance to botulism. Schreiber (39) in Prussia in 1884, and Hirschfeld (40) in Pomerania in 1885, reported cases in which the poisoning had been produced by herrings which had been cooked and then preserved in vinegar. Von Anrep (41) in 1886, Schmidt (42) in 1890, and Arustamoff (43) in 1891 reported outbreaks in Russia in which the poisoning was caused by smoked sturgeon, and Tschernyschew (44) in 1889 in Russia, and David (45) in 1899 in Germany, reported cases which followed the eating of smoked herring. Arustamoff also recorded a series of cases which were poisoned by eating preserved salmon, and Fischer (46) in 1897 described an outbreak which followed the ingestion of crabs.

In all these cases of "*Fischvergiftung*" the symptoms were apparently identical with those which occur in true botulism, and many authors, including Mayer, identify this type of fish-poisoning with botulism. However, there has been considerable discussion as to whether this should be done. Schlossberger (13),

1852, believed that fish-poisoning and sausage-poisoning were similar but not identical. Böhm (47), 1876, agreed with him. Erben (48) stated that the two conditions are apparently identical, but Esmein (49) does not think there is sufficient proof to establish identity. The question has apparently been settled by Madsen (50) in Denmark, who isolated a bacillus from poisonous fish which was identical with *B. botulinus*, thereby establishing that a certain type of fish-poisoning and botulism are identical.

Various other foodstuffs have been held responsible for poisoning of this type, but it is doubtful whether in all, especially in the earlier cases, the condition was really botulism. Kerner (27), 1824, and Siedler (51), 1827, recorded cases in which the poisoning was caused by the eating of old goose fat, and Kerner referred to another case in which smoked fat beef was responsible. Krugelstein (52), 1839, believed that butter, cheese, lard, smoked goose, flounders, and eels could produce the poisoning. Homans (53), 1871, referred to cases which had been produced by eating partridges, and Cohn (54) described one which followed the eating of rabbit paste which had been preserved under fat. Senckpiehl (15), 1887, and Wosnitza (16), 1909, described cases in which the liver of venison was at fault, and the latter author noted that persons who ate the heart of the same animal were not affected. Senckpiehl also recorded a case in which the symptoms followed the ingestion of stuffed goose, and Quincke (55), 1885, described one in which home-canned duck was responsible.

The most interesting outbreak, in view of the findings recorded in this report, occurred in Darmstadt in 1904 (56, 57), in which it was shown that the poisoning was caused by a salad which had been prepared from home-canned white beans. This is the only record in the European literature of the production of true botulism by a foodstuff of other than animal origin, and it was suggested by Landmann (58) that some pork must have been cooked with the beans, as otherwise the toxin of botulism could not have been formed.

Observations which are of interest in view of the present day knowledge of botulism were noted by many of the earlier investigators of this condition. Kerner (7), 1820, emphasized the fact that sausages in which the casings were incompletely filled did not become poisonous, and he concluded that exclusion of the air was necessary for the formation of the poison. He also noted that sausages which were packed in large casings, in which thorough smoking was difficult, were more apt to become poisonous than those which were packed in small casings. Schlossberger (13), 1852, also referred to this fact and noted that the poison was most often found in the center of the large sausages "wo der Abschluss der äusseren Luft am ausgesprochensten ist." Kerner also noted that the odor of the poisonous sausages was not putrid as in ordinary putrefaction, and Schlossberger recorded that it had a peculiar cheese-like odor. Kerner, Weiss (9), and others noted that the poison might be confined to certain portions of the sausage and that there were peculiar cheese-like, crumbly areas which they believed to be the poisonous portions. The taste was variously described as sour, bitter, and burning. Kerner suggested that it was possible that cooking

the sausage might inhibit the action of the poison, and Jaechnichen (38), 1850, recorded a series of cases in Russia in which those who ate the smoked fish without cooking became ill, whereas those who cooked the fish remained well.

Theories as to Etiology.

Numerous theories as to the origin of this poison in sausages and meats have been advanced by different authors. Perhaps the most fantastic theory was that of Wunderlich (59) who recalled that in mythology it is recorded that when slaves were tortured to death in the arena, their saliva became poisonous by the formation of *aqua toffana*, a substance which developed during the period of fear and suffering which preceded death. Wunderlich stated that the method of killing hogs in Würtemberg was exceedingly cruel and slow, and he suggested that there might be some poisonous substance produced in the saliva which was analogous to *aqua toffana*, and that in some way the flesh of the animal might become contaminated with this substance. It was believed at first that the poison might be due to contamination from lead and copper vessels in which the sausages had been cooked (60), but in 1793, when thirteen persons became ill and six died after eating "*Schweinsmagen*" or "*Blunzen*" at Wildbad, the attending physician was so impressed with the resemblance to belladonna-poisoning that he wrote:

"Dass die an und für sich schädliche Blunze einen krankmachenden Einfluss auf die gesunden Personen aufgeübt habe, der tödliche Ausgang aber durch eine beträchtliche Gabe Belladonna (man habe sie nun im Aufguss in die Wurst oder in die Brühe aus Unvorsichtigkeit oder Bosheit gethan) verursacht worden sei" (4).

In 1799 another outbreak occurred in Hofe Mosburg in which five persons became ill and two died, and, as a result of an investigation, the son of the family was accused of having poisoned the sausage with henbane seeds (61). In 1802 Jaeger (5), in the official warning which was published at Stuttgart, stated that chemical examination of the poisonous sausages had failed to show any evidence of the presence of mineral poisons, and he suggested that the toxic action of the sausages must have been due to the presence of some vegetable poison, probably to some poisonous seeds which had been mistaken for spices.

The first comprehensive discussion of the subject was published by Kerner in his two monographs which appeared in 1820 (7) and 1822 (8) respectively. The rapid increase in the number of reported cases had demonstrated that the poisoning was not due to any accidental or deliberate contamination with mineral or vegetable poisons, but that it depended upon the formation of some poison within the sausage itself. The chief interest of Kerner's work lies in the fact that he was the first to apply systematic, experimental investigation to the solution of the problem. After it had become evident that the poison was formed within the sausage, various theories were advanced, among which were two which seemed to be the more generally accepted; *viz.*, that prussic acid and pyroligneous acid had been formed within the sausage.

Emmert (62) had shown that prussic acid could be demonstrated in putrefying blood which was taken from cadavers, and he suggested that the blue-black color of the blood in the bodies of persons who had died from sausage-poisoning might be taken as evidence that the poisoning had been produced by prussic acid. Kühn (63) also held this opinion, for he wrote:

"Da die Blausäure aus Blutlauge erhalten werden kann, so war die Vermuthung, das der in den verdorbenen Würsten sich so schädlich äussernde Giftstoff nichts anderes als Blausäure sei, sehr natürlich."

However, chemical examination of poisonous sausages and of the blood and tissues of victims of sausage-poisoning failed to show any evidence of prussic acid (Kerner, Weiss, and others), and Rühle (64) wrote:

"Vorstehende Analyse beweist, dass diese Würste weder Blausäure, noch Metallgifte enthalten, ob aber der der Gesundheit nachtheilige Stoff in der in diesen Würsten augenscheinlich enthaltenen Säure liege und ob dieselbe von einer eigenthümlichen Beschaffenheit sei, das wäre noch durch weitere chemische und medicinische Versuche zu bestimmen."

Kerner undertook experimental investigation to determine this point, and he showed that the symptoms and signs of prussic acid-poisoning are quite unlike those of sausage-poisoning, especially in the length of time which elapses before the beginning of the symptoms, the long course, and the absence of odor in the cadaver. He mentioned, however, that the symptoms of sausage-poisoning more closely resemble those of prussic acid-poisoning than those of such plant-poisoning as is produced by belladonna in that there is a complete absence of mental symptoms.

The theory that sausage-poisoning was due to the presence of pyroligneous acid was based upon the experiments of Cormack and Corneliani (65) who found that it was present in the smoke of certain kinds of wood. It was thought that during the process of smoking there was an accumulation of the acid in the sausages, and the theory was apparently substantiated to a certain extent by the fact that analysis of the poisonous sausages had always shown the presence of some acid.

Kerner (7) recorded that a colleague had pickled meat by immersing it in crude acetic acid and had then smoked it. One person who had eaten some of the meat had suffered from vomiting and diarrhea, but had recovered, and another had shown no symptoms. However, a cat had also eaten a portion of the meat, and it died within a few hours. He also recorded experiments on rabbits and cats in which he noted the symptoms and pathological findings after poisoning with crude acetic acid. He found that herbivorous animals withstood much larger doses than carnivorous animals, and he concluded that poisoning with crude acetic acid produces symptoms and pathological conditions which are somewhat similar to those produced by sausage-poisoning, although they are not identical.

Kerner noted that the majority of cases of sausage-poisoning occurred in the spring months, especially in April, at a time after there had been alternate spells of freezing and thawing, and that sausages which had been prepared in the fall, and had been subjected to alternate freezing and thawing, were especially liable to undergo putrefaction, even though they had been boiled during the process of preparation. He conducted an elaborate series of chemical investigations of these sausages by various methods of distillation, and succeeded in isolating a fatty acid substance which was extremely toxic for animals, and which produced dysphagia, aphonia, and paralysis, symptoms which were analogous to those of sausage-poisoning. He was also able to produce the same fatty acid substance, which he called "*Leichensäure*," from rancid fat and from the blood of cadavers.

At first Kerner (8) believed that this "*Leichensäure*" was the toxic agent of sausage-poisoning, but in 1823 Jaeger (66) expressed the opinion that the fatty acid was not the toxic agent in the sausage, but that there was some nitrogen-containing substance, such as picric acid or "Welther's Bitter," which was the poisonous substance. Kerner (27) then modified his view to the extent of considering the fatty acid element an essential constituent which was closely related to some nitrogen-containing substance, probably an alkaloid analogous to Welther's Bitter. To substantiate this theory he pointed out that in such plants as *Veratrum* the active principle consists of an alkaloidal substance which is closely associated with certain fatty acids.

Kerner emphasized the point that the change which led to the formation of this fatty acid complex in sausages was not identical with ordinary putrefaction. He noted that it was never found in sausages in which the casing was incompletely filled, or in those which had not been boiled, and he assumed that for its formation it was necessary that the fats should be cooked, possibly incompletely, and that they should be excluded from the action of the air. He also stated that if putrefaction subsequently occurred, the presence of the sulfur dioxide gas would lead to a further reduction of the fatty acid complex into its simpler, non-toxic constituents.

In 1824 Weiss (9), one of Kerner's students, reported a series of cases in which he had made the observation that in the vomitus of the patients, and in the contents of the stomachs and intestines of the cadavers, there was a peculiar yellow substance which had a bitter taste, and which was not bile. In an extract of the poisonous sausage, prepared by placing it in boiling water and allowing it to stand for several days, a scum of a similar yellow substance collected on the surface. Weiss believed that this substance was adipocere which had been formed in the sausage, but Kerner, in a foot-note, stated that it was probable that had chemical analysis been made, it would have shown that the yellow substance was a combination of Welther's Bitter and fatty acid. Weiss agreed with Kerner that the toxic agent was probably a fatty acid with a closely associated alkaloid, but he believed that the action of the gastric juice was necessary before it was rendered poisonous.

Various investigators attempted to confirm the observations of Kerner, but without success. Buchner (67) conducted a series of investigations with a poisonous sausage and with fresh lard, but failed to obtain any toxic product of distillation. He succeeded in isolating a combination of acetic acid and an ethereal oil to which he gave the name "*Pyrofettäther*," but Kerner had also obtained a similar substance which he called "*Fettsäures-Fett*," and which was different from his "*Leichensäure.*" Kastner (68) also made chemical analysis of sausage and concluded that "*Wurstsäure*" may be an intimate combination of acetic acid or lactic acid with some of the products of putrefactive fermentation of animal material, which are themselves poisonous in small doses. Horn (26), 1828, reported a series of experiments which he had made in collaboration with Schrader, a chemist, in which they had attempted to produce an acid substance from lard, but without success. He concluded his discussion of the subject by saying "that he cannot concur in any of the opinions put forth as to the nature of this poison; and that it appears to him to be some matter developed by spontaneous putrefaction, and that this spontaneous putrefaction arises perhaps in improperly made farcimina."[2]

Various other authors advanced theories from time to time. Dann (10), 1828, believed that the poisonous material did not necessarily reside in an acid, but was probably due to an "*Empyreumatisches Oel*," acrolein, or *acrol*, which was not toxic in the pure state, but became poisonous when combined with various fatty acids. Bodenmüller (69), 1834, noted that there had never been any poisoning from "*Leberwurst*" which had not been smoked, and which had been prepared during the winter months, and he concluded that the formation of the poison must depend upon the method of smoking and heating the sausages. Krugelstein (52), 1839, agreed with him. Tritschler (70) believed that the active principle of the poisonous sausage was not a pure chemical compound. Lussana (71), 1845, ascribed the poisoning to the presence of creosote which was formed during the process of smoking the meat, by its combination with the crude acetic acid in the smoke. Liebig (72), 1843, believed that the toxic action was due to the action of a ferment, but Schlossberger (13), 1852, argued that this could not be true, and suggested that it was due to the presence of an organic base belonging to the group of alkaloids.

Heller (73), 1853, was the first to suggest that the poisonous material might be due to the growth of a microscopic vegetable organism within the sausage, and he described a phosphorescent, fatty growth or mould which he believed to be responsible for the formation of the poison. Van den Corput (74), 1855, believed that it was due to a low form of plant life, a mould or alga, which he called "*Sarcina botulina.*" Wittig (75), 1856, and Kasper (76), 1858, also believed that a mould was at fault.

Subsequent to Zenker's discovery (77) in 1860 that *Trichina spiralis* is responsible for a characteristic symptom complex in man, various authors advanced

[2] Arrowsmith (26), p. 39.

the theory that sausage-poisoning is but a type of trichinosis. However, Virchow (78), 1864, and Husemann (79), 1866, showed that the two conditions are etiologically distinct; and Rupprecht (80), 1864, and Müller (81), 1870, published elaborate tables of differential diagnosis.

No new theory of importance was advanced until 1886 when von Anrep (41) reported the isolation of a fixed base from the flesh of a sturgeon, to which he gave the name "*Ptomato-Atropin.*" He found that the base could be separated by precipitation, and that it was extremely toxic for rabbits. He believed that this was the toxic substance of poisonous sausages. In the same year Ehrenberg (82) had isolated choline, neuridine, dimethylamine, and trimethylamine from a poisonous sausage, and he believed that the choline and neuridine were responsible for the poisoning, although he was unable to reproduce the characteristic symptoms in rabbits with either. Nauwerck (83), 1886, announced that putrefaction of a type which could produce the substances which Ehrenberg had described, must be of bacterial origin, and he reported the isolation of three forms of bacteria which he had isolated from the same sausage. One of these was a bacillus which liquefied gelatin and caused rapid putrefaction of sterile blood. An apparently identical bacillus was isolated from the intestinal contents of a hog by Redner (84), and Nauwerck believed that it was the true cause of the poisoning. He explained the late appearance of the symptoms by assuming that the bacilli were introduced into the intestinal tract with the sausage, and that they produced putrefactive changes in the intestine, which, in turn, led to an autointoxication.

The actual cause of botulism was demonstrated by van Ermengem (34, 85, 86, 87) who studied a series of cases which occurred at Ellezelles, in Belgium, in 1894. In the Ellezelles outbreak twenty-three persons became ill, and three died, after eating ham which had been preserved in brine. From portions of the ham and from the spleen and intestinal contents of one of the victims, van Ermengem succeeded in isolating a Gram-positive, spore-bearing, anaerobic bacillus to which he gave the name *Bacillus botulinus*. He found that infusions of the macerated ham, and bouillon cultures of *Bacillus botulinus*, produced the typical symptoms of botulism in guinea pigs, rabbits, cats, pigeons, and monkeys. The bacillus itself is a saprophyte, and the poisoning is due to a toxin which is formed when it is grown under anaerobic conditions.

Van Ermengem's observations have been confirmed by various authors. In 1900 Römer (35) isolated a similar bacillus from ham which had caused the illness of four persons. In 1901 Madsen (50) recorded the recovery of a bacillus from fish which had caused the illness of three persons, and he stated that the toxin of his strain was neutralized by the antitoxin of van Ermengem's strain. In 1904 Landmann (58) demonstrated *Bacillus botulinus* in the beans which had caused the poisoning in the Darmstadt outbreak, and in 1906 van Ermengem recovered another strain from ham which had poisoned eight persons in West Flanders. In 1913 Ornstein (88) reported that he had recovered the bacillus from ham which had caused the death of two persons, and from the spleen of

one of the victims; and in 1913 Schumacher (18) reported that he had recovered the bacillus from ham which had caused the illness of six.

Mortality.

The mortality of botulism has varied greatly in different outbreaks. In individual instances it has been extremely high, in some 100 per cent, but in others it has been correspondingly low. Wosnitza (16) recorded a series of fifty-nine cases of which only four died. In Kerner's (7, 8) series of 159 cases there were 84 deaths, a mortality of 52.8 per cent, and in Schlossberger's series (13) of 400 cases there were 150 deaths, a mortality of 37.5 per cent. The most complete collection of cases is that of Mayer (25) in 1913, in which he reports 812 cases of which 365 were fatal. The mortality of Mayer's series was 44.9 per cent.

AMERICAN LITERATURE.

A review of the American literature reveals the fact that very few cases of botulism have been recognized in this country, but in a survey of the available reported cases of food-poisoning during the past 25 years it was found that there have been a number of cases in which the symptoms are more or less indicative of this condition.

In 1894 Seelye (89) reported an outbreak of food-poisoning in which fifteen persons were affected after eating turkey at Thanksgiving dinner. The chief symptoms were dizziness and disturbance of vision, and it is noted that the pupils of all the patients were widely dilated. He believed that the poisoning was due to belladonna, stramonium, or hyoscyamus which had been eaten by the turkey, but Herzog (90) drew attention to the fact that all the symptoms could be explained on the basis of ptomaine poisoning, and that this was a more probable explanation of the poisoning. The rapidity of the onset of symptoms, about three-quarters of an hour, and the rapid recovery practically exclude a diagnosis of botulism, although the initial symptoms were somewhat suggestive.

In 1898 Spiller (91) reported a case of "neuritis from the ingestion of putrefying pork." The initial symptoms in this case were soon followed by dizziness so severe that the patient was unable to stand. Symptoms of a polyneuritis soon developed and persisted for over 2 months.

The clinical course of this case does not suggest botulism, but the author refers to the ganglion cell lesions of botulism as indicative of the changes that may be produced in the central nervous system by food-poisoning, and he suggests that similar lesions in the ganglion cells of the spinal cord could explain the symptoms of his case.

In 1899 Lewis (92) (Southern California) reported a case of poisoning after

eating a beef tamale. Several hours after the meal the patient became ill, had a severe headache, and soon developed vomiting and purging which persisted for more than 24 hours. Ptosis, photophobia, deafness, and dysphagia followed, and there was a diminution of "all the secretions." There was extreme prostration and a tedious convalescence, and for a time there was paralysis of the rectum and bladder.

A number of unusual symptoms were noted in this case, such as a persistent high fever for several weeks, a hyperemic blush of the skin of the trunk, swelling of the joints, and a coarse scaly rash on the body. During the 4th week of the illness there was coma and opisthotonos for several days. The author states that typhoid fever was excluded by the type of fever and the laboratory tests.

There is some doubt as to whether this case was one of botulism complicated by an intercurrent infection, a combination of events which is not unknown, but the type of onset and the early course of the disease are strongly indicative that this was the case.

In 1902 Jellinek (93) (San Francisco, California) reported seven cases of botulism after eating "tainted" boiled beef. Three of the patients died. The symptoms of one fatal case were outlined in some detail as follows: The patient became ill on December 3, 6 days after the meal, and complained of lack of appetite, dryness and scratching in the throat, and great prostration. On the following day there was dryness of the mouth and throat, extreme thirst, ptosis, diplopia, inability to swallow, choking whenever attempts were made to swallow, retarded movements of the tongue, slow, hoarse, nasal voice sounds, and inability to raise the head. At this time there was a fever of 102°F., a pulse rate of 120 to 140, and evidence of consolidation in the lungs. Sensation was undisturbed throughout. There was obstinate constipation and a small amount of albumin in the urine. Death occurred 10 days after the meal.

Necropsy was performed by Dr. Ophüls who recorded marked rigor mortis, hyperemia and edema of the meninges with much dark blood in the basilar sinuses, hyperemia and edema of the cerebellum, hyperemia of the plexuses and basal ganglia, bilateral hydrothorax, injection of the pericardium, petechiæ in the pleura, hyperemia, edema, and bronchopneumonic patches in the lungs, enlargement of the spleen, passive congestion of the liver, kidneys, and mucous membrane of the stomach, and hyperemia of the mesenteric and retroperitoneal lymph glands. Complete record of the histologic examination is not given, although it is noted that there was engorgement of the capillaries of the medulla, pons, and cerebellum.

In 1904 Anderson (94) described a case in which nausea, dizziness, and incoordination of muscular movement persisted for 6 weeks. The suspected meal consisted of soup, beefsteak, sweet potatoes, and dessert. The clinical picture in this case is not typical of botulism, although there are some points in which it is suggestive.

In 1905 Bryant (95) attempted to explain the onset of deaf-mutism in two children by assuming that it was due to food-poisoning. The record of the clini-

cal findings is incomplete and does not suggest botulism, but the author believed that the deafness was due to an involvement of the nuclei of the auditory nerves which was produced by the food-poisoning in a manner similar to that which had been described by Marinesco, Kempner, and others in cases of experimentally produced botulism.

In 1907 Sheppard (96) (Ontario, California) recorded an outbreak of food-poisoning in which three persons became ill, and two died after eating canned pork and beans. The onset of symptoms occurred in from 36 hours to 4½ days after the first meal of the suspected food. Twelve chickens became ill, and nine died after eating the remains of the food.

The symptoms were practically identical in all the cases. There was an absence of pain, fever, gastrointestinal, and sensory disturbances. The first complaint was dimness of vision; the party was on a shooting trip and one of the men noted that he could not shoot straight, and another that he could not hit a stick of wood which he was attempting to chop. There was early ptosis, diplopia, and difficulty in swallowing. Later there was progressive muscular weakness "with gradually developing motor paralysis," inability to swallow, inability to raise thick mucus from the throat, and marked constipation. The urine secretion was normal, the pulse showed little change except irritability when movements were attempted, there was no fever, and the mentality was clear. Incoordination of movement was so great that the writing was illegible. There is no record of necropsy findings.

In 1910 Peck (97) (Sawtelle, California) described an outbreak in which twelve persons were ill and eleven died after eating home-canned pears which "were not exactly sour but tasted sharp and bit or tingled the tongue and throat." The only person who recovered had merely tasted the fruit and had swallowed a very small amount.

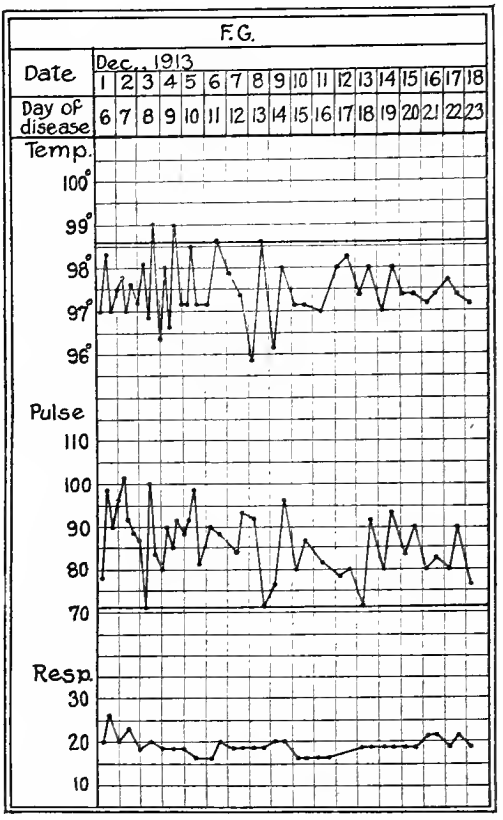
The symptoms were alike in all the cases, including the one which recovered. There was marked incoordination of movement and muscular weakness. The tongue was thickly coated and could be moved with difficulty. There was dimness of vision, dilatation of the pupils, and disturbance of the pupillary reflexes. The pulse was rapid in the only case in which it was recorded, the temperature was subnormal, speech was difficult, and there were choking spells when the patients attempted to swallow. There was initial nausea and vomiting, and constipation was persistent. Mentality was clear throughout.

The average length of time which elapsed between the taking of the food and death was 40 hours, the shortest time being 25 hours, and the longest 63 hours. There is no record of necropsy findings.

There was some difference of opinion as to the real cause of the poisoning in this outbreak, as some of the victims had also eaten tamales, but it was definitely established that the only article of food of which they all had partaken and which was at all open to suspicion was the spoiled fruit.

In 1913 Stiles (98) (Boston, Massachusetts) recorded his own illness which followed a meal in which the suspected food was minced chicken. About 2

hours after the meal he "became conscious of growing inertia" and "of a curious hyperesthesia of the pharynx." He was faint and unable to stand. About 4 hours after the meal he became nauseated and vomited portions of chicken which tasted "rough, rancid, or acid." Nausea, vertigo, and nystagmus occurred



TEXT-FIG. 1. Temperature, pulse, and respiration curves of Miss F. G., one of the patients of the Stanford University series. The patient was taken to the hospital on the 6th day after she had eaten the string bean salad. She was very ill for the first few days, sat up for the first time on the 19th day, and was allowed to go home on the 23rd day.

whenever he raised his head. On the following day there was extreme vertigo if he moved his head, vomiting of undigested chicken, nystagmus, and cardiac irregularity. Evacuation of the bowels was obtained with an enema and there was resumption of urine secretion. For a few days there was a gradual return

of strength and the patient walked half a mile on the 6th day after the meal, and visited his laboratory on the 7th, but during the night of the 7th day he awakened to find that the prostration and vertigo had returned and that he could scarcely speak. On the following day there was difficulty in talking and swallowing, and food was regurgitated through the nose. Thick mucus collected in the pharynx and was removed with difficulty. The attacks of nystagmus became less frequent but diplopia developed, and there was great prostration.

Recovery was extremely slow and tedious. The persistent symptoms were constipation, burning in the throat, ataxia, and disturbances of vision. The left pupil was smaller than the right and there was some failure of accommodation.

In 1914 Wilbur and Ophüls (99) (Stanford University, California) reported a series of twelve cases which developed after eating salad which was prepared from home-canned string beans. The symptoms of all were typical of botulism but varied greatly in degree of severity. One of the patients died.

The following record of the clinical and autopsy findings of the fatal case is quoted from their report.

"Young healthy woman, aged 23, on the second morning following the ingestion of the meal at which time beans were taken, complained that objects were often seen double. There was no headache or vomiting; urination apparently as usual. A laxative had been taken because of constipation and the bowels had acted. On the fourth morning the patient was unable to eat because of the inability to swallow. There was no pain. Later on in the day she swallowed with difficulty. On the sixth morning her mouth was dry, there was considerable thirst, no abdominal colic, no appetite, marked asthenia, with the muscular power in the left arm less than normal. Voice was slightly nasal with inability to articulate distinctly. Temperature was 97.8° to 99° F., pulse 104. There was ptosis of both upper lids; both pupils were dilated but reacted to light, with normal fundi. The breath was foul, tongue coated, and it and the pharynx covered with sticky, whitish, viscid mucus. There was considerable edema of the uvula.

"The condition did not change very much in the next few days except that the patient felt very weak, was unable to raise the head without help, at times would swallow well, at other times was unable to take the simplest food, and the pulse became more rapid and dicrotic. Blood pressure was normal. At one time she became quite talkative and complained that her jaws did not open naturally and she could not see well, but for the most part she was quiet and slightly depressed.

"On the tenth day she brought up with very much difficulty some mucus which had been interfering greatly with her throat and had a period in the afternoon when she was unable to swallow, had difficulty in breathing, and cold extremities. Later on she was able to take an egg-nog, but complained of a spontaneous choking sensation. In the night to the distress of these attacks was added an inability to breathe through the nose.

"On the twelfth day involuntary movements of the bowels occurred. Some relief from the choking, breathless sensation was gained by swabbing the throat. The patient vomited some dark green fluid after a large soft involuntary stool and began to feel very warm and excited, axillary temperature going up to 100.4°. After a period of chills, great restlessness, choking sensation, increasing temperature, and higher pulse rate, together with the signs of hypostatic pneumonia, the patient had a collapse from which she recovered on the administration by Dr. Williams of an intravenous transfusion of normal salt solution.

She vomited later a considerable amount of blood and although her breathing became more superficial, her weakness more evident, the sensorium remained perfectly intact" (Text-fig. 2).

"On the thirteenth day she died following a respiratory disturbance apparently due to sudden paralysis of the diaphragm, since the heart continued to beat for some time. Various forms of stimulant and other treatment were administered without avail. The necropsy, performed by Dr. William Ophüls, was practically negative except for areas of collapse and bronchopneumonia in the posterior parts of the lungs, and certain lesions of the central nervous system, which will be described later."

Necropsy.—"The brain in our case was obtained within twenty-four hours in a very good state of preservation.

"The gross findings at necropsy were entirely negative except for a moderate hyperemia of the cerebral vessels, which was especially pronounced at the base of the brain and in the region of the fourth ventricle, where there also was found some edema. No hemorrhages could be made out with the naked eye on several sections through the brain stem. The internal organs were markedly hyperemic, which was especially noticeable in liver, kidneys, and intestines. This general hyperemia has been a constant finding in all cases of human botulism and is also found in the experimental disease in animals. The dilatation of all the blood vessels was also very evident in sections from all organs examined. Typical bronchopneumonic lesions were found in the lungs, which condition probably accelerated the death of the patient.

"The following is a short description of the microscopical findings in the brain:

"The brain stem was sectioned transversely at different levels at the time of the autopsy and carefully hardened in frequently changed absolute alcohol. Numerous sections were made from all levels and special attention paid to the regions of the more important nuclei. Sections were obtained from all motor and some of the sensory nuclei on both sides. The nuclei studied were those of the oculomotorius (third), trochlearis (fourth), trigeminus (fifth), abducens (sixth), facialis (seventh), acusticus (eighth), glossopharyngeus (ninth), vagus (tenth), and hypoglossus (twelfth) nerves. The following lesions were found:

"Thrombosis of the right arteria vertebralis at lower end of the medulla and extending into the arteria basilaris some distance. The middle part of the arteria basilaris is free. The anterior portion of it again is filled with thrombus. There are thrombi in a few of the pial branches of the basilar artery. Many pial veins at the base of the medulla and pons are filled with thrombi, more especially on the right side, and there are several thrombi in the veins of the tela chorioidea above the corpora quadrigemina and the posterior part of the third ventricle. All blood vessels in the brain tissue are much distended and full of blood. Both in the ventral and in the dorsal parts of the brain stem one finds quite a few thrombosed blood vessels, some of which are situated near important nuclei; for instance, one was found near the right vagus nucleus, several in the nuclei of the olives, several in the substantia ferruginea, several in the right nucleus trochlearis, several near the right nucleus of the oculomotorius, and similarly several near the left nucleus oculomotorius, but somewhat further away from it. Several perivascular hemorrhages were encountered quite commonly about thrombosed veins and others. The thrombi in all places were very rich in polymorphonuclear leukocytes, but no bacteria could be demonstrated in spite of the use of various staining methods, including Weigert's modification of Gram's method. All thrombi were very rich in fibrin and contained comparatively few masses of conglutinated blood platelets" (Fig. 2).

"The Nissl granules stain unusually well in all sections especially in the large motor

ganglion cells. They are quite normal in size and arrangement. Nothing unusual could be detected in spite of especially careful study of them. The nuclei of the ganglion cells are perfectly normal.

"Sections were also taken from various parts of the cortex of the cerebellum. There was no difference in the appearance of the ganglion cells from these other regions. Few thrombi were seen in the pial veins. The blood vessels in the cortex are dilated and full of blood, but none show thrombosis or perivascular hemorrhages.

"No thrombi were found in any other organs in spite of the study of many sections except one in a vein in the submucosa of the intestine and the very beginning of the thrombotic process in few of the dilated vessels in the ovarian cortex.

"Anaerobic cultures were made from the intestinal content both of the small and large intestines, from the cerebrospinal fluid, from the heart blood, and from the spleen. The cerebrospinal fluid and the heart blood were sterile and the *Bacillus botulinus* was not found in any of the other cultures."

Dr. T. M. Williams (100) (Palo Alto, California) treated seven of the patients of the Stanford University series, and reported his observations at the annual meeting of the California State Medical Society in 1914. It is unfortunate that his report has not been published. The following brief description is abstracted from his manuscript.

The initial symptoms developed in from 1 to 4 days after the poisonous food was eaten. Two of the patients were not seriously ill; one had attacks of vomiting, and the other diarrhea, but showed no other symptoms. They did not consult a physician. In Williams' series, disturbance of vision was the initial symptom in four, difficulty in swallowing in one, rapidly progressing weakness in one, and nausea, vomiting, and abdominal pain in the other. One died on the 14th day after eating the poisonous food, two were discharged from the hospital on the 21st day, three were discharged on the 23rd day (Text-fig. 1), and one was unable to leave until the 40th day. All the surviving cases were apparently well in from 4 to 8 weeks, and had returned to their University work within 2 months. It is noted that 15 months later none showed any ill effects from the poisoning.

The symptoms and course of the illness were practically the same in all the cases, varying only in severity. There was blepharoptosis, mydriasis, diplopia, strabismus, and nystagmus. The dilated pupils reacted sluggishly to light stimulation, and accommodation was impaired. Some of the patients complained of dimness of vision but there were no demonstrable lesions in the retinas. In only one of the cases were disturbances of vision absent. There was a peculiar expressionless appearance of the face, and the jaws could not be opened more than one-half to three-quarters of an inch. The breath was fetid, the tongue heavily coated, and the walls of the pharynx covered with tenacious, glairy mucus. In one case there was marked edema of the glottis. The voice was nasal in character and articulation was indistinct. There was difficulty in swallowing, and in the more severe cases there was regurgitation of food through the nose. The pharyngeal muscles and the soft palate were partially or completely paralyzed in all the cases. The patients experienced great difficulty in

clearing the mucus from the pharynx. There was a continuous sensation of fullness in the throat and of choking, and the patients complained bitterly of the difficulty in breathing. One patient was so fearful that respiration would cease unless he maintained voluntary respiratory efforts, that he dared not go to sleep, and all the more severe cases were conscious of impending suffocation.

A striking feature of all the cases was the progressive muscular weakness. This was especially marked in the muscles of the head and neck. Several of the patients complained that they were unable to masticate their food.

"There was extreme flaccidity of the muscles of the neck, allowing the chin to rest upon the chest, or the head to fall backward if not supported when the patient was elevated. It was a common occurrence to see a patient move her head with her hands, and one girl who had long braids used them, with the head of the iron bed as a pulley, to swing her head into position when she wished to move. The head could be rotated and held from falling to either side, but the anterior and posterior supports were lacking."

There was a general muscular relaxation and an appearance of fatigue that was most distressing. The reflexes in the extremities were normal.

Mentality was unimpaired in all the cases. The pulse varied from normal to over 130 per minute, and the blood pressure was normal or slightly lower than normal. The temperature tended to be subnormal, except in the last stage of the illness. The excretion of urine was unimpaired, except in one case, and the blood picture was unchanged. Obstinate constipation was noted in all the cases.

Williams noted that there was a most striking variation in the intensity of the symptoms within short periods of time. It was not unusual to find a patient, who a few hours previously had seemed extremely ill, complaining of great respiratory distress and unable to raise the eyelids, able to swallow and talk without difficulty, and to breathe comfortably. He added: "Had I other cases to treat, I should be guarded as to my prognosis, and I should not be too much elated or discouraged by sudden variations."

In 1915, Frost (101) (Los Angeles, California) recorded three fatal cases of botulism. In two it was probable that the poisoning was due to the ingestion of old "*Wienerwurst*," but in the other the cause was not established. There was initial weakness, dizziness, and disturbance of vision. In one there was vomiting. There was difficulty in swallowing and in enunciation, and the tongue was swollen and heavily coated. Marked weakness, diplopia, and strangling followed. In two cases death occurred from "inanition," and in the other during an attack of strangling. The temperature was subnormal in all, and the pulse was rapid and weak. In one case there was evidence of a terminal bronchopneumonia.

In 1916 Lancaster (102) (Boston, Massachusetts) reported a case in which the source of poisoning was not determined. The initial symptom was diplopia, but was soon followed by vertigo, ptosis, complete ophthalmoplegia, and fixed eyeballs. There was no dilatation of the pupils but reaction to light was sluggish.

The appearance of the fundi was normal. Nausea and vertigo were made worse by movement, but were less severe in the dark. There was moderate dysphagia, difficulty of speech because of impaired movement of the tongue, tremor of the hands, and marked weakness. There was no disturbance of secretions. Facial paralysis developed and persisted for 6 weeks. Mentality was clear although there was persistent somnolence. Recovery was slow and tedious, disturbance of vision, incoordination of muscular movement, and vertigo being the persisting symptoms.

There were several unusual symptoms in this case, a fever which reached 102° and which subsided by lysis, and persistent diarrhea being the most important. Nausea persisted for weeks. The leukocyte count was 7,000, the systolic blood pressure was 120 mm., and the urine was normal.

The most recent report in the American literature appeared in February of this year, when Curfman (103) recorded seven cases, five of them fatal, which had occurred in Colorado several years ago. The poisoning was apparently caused by canned string beans or canned spinach, and the outbreak is of considerable interest in that it is the first recorded instance in which the poisonous vegetables were prepared in a regularly equipped canning factory. It is also of interest that the vegetables were canned in Kansas, as proof is thereby established that *B. botulinus* is to be found in the middle western states.

The onset of symptoms in Curfman's cases varied from a few hours to 4 days after the poisonous food was eaten. Three of the patients complained of initial gastrointestinal disturbances, nausea, vomiting, and abdominal distress, but in the four remaining cases the symptoms of central nervous system involvement were the first noted. In one case the nausea and vomiting occurred 3 days after the appearance of dizziness and visual disturbance. The symptoms were very similar in all the cases. There was dizziness, some headache, diplopia, ptosis, dilatation of the pupils, diminution of reaction to light, paralysis of accommodation, difficulty in swallowing, difficulty in talking, and eventually complete aphonia in the more severe cases, marked general weakness, and constipation. The patients complained of the accumulation of glairy mucus in the throat and of dryness in the mouth. They strangled when they attempted to swallow. Mentality was clear throughout and there is no record of any sensory disturbances. It was noted in two cases that the temperature was subnormal, and, in one of these, that the pulse rate was above 100 per minute. Two of the patients died within 48 hours, one on the 3rd day, one on the 6th day, and one 2 weeks after the poisoning. Death occurred from respiratory failure.

Of the two patients which recovered, one was apparently a very mild case but the other was more seriously ill. The latter patient recovered very slowly; she was unable to comb her hair for 4 weeks, or to walk without assistance for 6 weeks. She suffered much from dyspnea on exertion, and "the heart action was weak and rapid." 3 months after the poisoning it was noted that the patient was "unable to read or do any close work," that she had frontal headache, was dyspneic on exertion, and was very constipated. The pulse was 96 per minute,

regular, and of fair quality. Muscular strength was gradually returning. The pharyngeal reflex was still sluggish but the knee-jerks were overactive.

An interesting feature of Curfman's report is the statement that five burros died after eating the garbage which contained the remnants of the meal. It is noted that they had "symptoms similar to those observed in the human cases."

REPORT OF NEW CASES.

Outbreak 1. Five Cases.

On Sunday, Mar. 7, 1915, five persons in Fallbrook, California, ate together at supper, which consisted of bacon, potatoes, cooked dried beans, bread, butter, and home-canned apricots which had been canned without sugar. The food was all apparently of good quality except the apricots which were moulded on the surface and discolored for about one-half the depth of the jar. The upper portion of the fruit was discarded, but the lower portion was served and was found to be somewhat bitter, though not unpleasantly so. On the following Tuesday three of the party were very tired, and some were dizzy and had transient double vision and headache. On Wednesday the three adults attempted to work but became exhausted and went to bed. Each took a large dose of sodium phosphate. On Thursday morning all were much worse and Dr. Charles Pratt of Fallbrook and Dr. Reid and Dr. Nichols of Oceanside were called, to whom I am indebted for the record of the cases. I have also made use of the Coroner's report which was published in *The San Diego Union* of Mar. 22, 1915.

When first seen, two of the three adults were almost in collapse, and the third was confined to her bed. All showed the same symptoms; *viz.*, ptosis, double vision, extreme dilatation of the pupils, dryness of the mouth and throat, accumulation of thick, viscid mucus in the throat, difficulty in swallowing, difficulty in talking, and extreme muscular weakness. There was no nausea or vomiting, no pain, and no sensory disturbance. Purgatives and enemata were given, and strychnine and atropine were administered hypodermically. Two of the patients died the same evening, the immediate cause of death being "paralysis of the respiratory tract." Consciousness was maintained to the last, and the heart continued to beat for several minutes after respiration ceased. There was marked terminal cyanosis.

On Friday it was noted that the mother was considerably better and that there was less disturbance of vision and swallowing, but on Saturday she exhibited the same symptoms as in the two previous cases, and died in the afternoon.

On Thursday morning the youngest grandchild, aged 5 years, was apparently in perfect health, although on the previous day he had become very tired while walking to his home, a distance of about a mile. During the afternoon of Thursday, however, he developed ptosis, double vision, and difficulty in swallowing, talking, and breathing. He died the following day. The elder grandchild, aged 9, showed the initial symptoms on Friday afternoon, and she died the following Thursday. The course in this case was much more prolonged, but the terminal symptoms were identical with those of the other patients (Text-fig. 3).

The symptoms were practically identical in all the cases, and were of the type seen in bulbar paralysis. There was no fever, no gastrointestinal disturbance and no mental or sensory disturbance. The pulse was rapid and of poor quality.

Necropsy was performed on the body of the elder grandchild, but "no information was obtained which throws any light upon the subject, as to the origin of the trouble."

A dog which ate part of the discarded apricots became paralyzed in the hind legs for a few days, but ultimately recovered. Several chickens, which also ate some of the apricots, became ill with limber-neck, and seven of them died. "Bacteriologic examination of the viscera and of the blood of one of the chickens revealed nothing of a pathological significance."[3]

There was some difference of opinion as to the cause of this outbreak, although all agreed that it was clinically botulism. Some of the investigators believed that the bacon was responsible for the poisoning, although they admitted that there was no evidence of spoiling in the portion that remained. Moreover, other persons had eaten portions of the same bacon without injury, and it is known that the chickens and the dog did not eat any of the bacon, although they did eat the portion of the apricots which was discarded.

Outbreak 2. One Case.

On Sunday, Oct. 17, 1915, Mrs. X., a housewife aged 35, opened several jars of corn which she had canned a few months previously. The contents of the first two jars were evidently spoiled and were thrown out. The corn in the third jar appeared to be good, but after she had tasted about two teaspoonfuls Mrs. X. decided that it was also spoiled. About 4 or 5 hours later she noticed a pain in the abdomen for which she took some laxative pills, and obtained an evacuation of the bowels on Monday morning. Later in the day she began to have difficulty in swallowing, and called in Dr. I. E. Barrett of Hillsboro, Oregon, to whom I am indebted for the following record.

The patient when first seen was very restless, almost hysterical, and complained of inability to get her breath, inability to swallow, and pressure about the throat. There was no history of nausea or vomiting, and no diarrhea. The mouth was dry. There was much severe cough without sputum, and the patient complained of difficulty in raising thick mucus from the pharynx. Swallowing

[3] The portions of the text which are in quotation marks are quoted from the County Coroner's report.

was at first difficult and later impossible. Articulation was poor, and, toward the end, the patient mumbled unintelligibly. There was no disturbance of mentality except the hysterical condition at the onset, and coma for a short time before death. The pulse was thready, from 100 to 120 per minute. There was no fever and the temperature on the last day was subnormal. The excretion of urine was normal. Respiration was extremely labored toward the end, and death occurred from respiratory failure.

Necropsy was done by Dr. Barrett. No cause of death could be found.

Several hogs and chickens ate the discarded corn. The hogs showed no ill effects, but fifty chickens became paralyzed and died. Through the courtesy of Dr. Leon W. Hyde of Hillsboro, the carcass of one of the chickens was sent to me. Putrefaction had progressed to such an extent that it was impossible to make histologic examination of any of the tissues, but from the contents of the gizzard an anaerobic bacillus was isolated, which is morphologically like *Bacillus botulinus*, and which produces an extremely virulent toxin when grown on suitable media.

Outbreak 3. One Case.

On Nov. 18, 1915, Mrs. H. opened some home-canned asparagus and noted that the contents of the jar "were a little sour," but, as was her habit, she drank the juice from the jar, and placed the stalks on the stove to cook. While the asparagus was cooking the odor was not good, and her son, after tasting a piece, threw it all out. About 6 hours after drinking the asparagus juice Mrs. H. became nauseated and vomited repeatedly. She was attended by Dr. F. P. Gundrum of Sacramento, California, who sent me the record of the case.

Dr. Gundrum first saw the patient on the 4th day of the illness, a short time before she died. There was dimness of vision, bilateral ptosis, strabismus, diplopia, and loss of pupillary reflex to light. The tongue was coated, speech was impaired, and there was difficulty in swallowing, decrease in the flow of saliva, accumulation of thick, tenacious mucus in the throat, and dryness of the skin and buccal mucous membrane. There was persistent constipation and almost complete suppression of the urine. There was marked general weakness and incoordination, especially of the muscles of the arms. The pulse was 108, of poor quality, and the temperature was 102°F. Death occurred from respiratory failure, 3½ days after the appearance of the first symptoms.

Necropsy was not permitted.

Outbreak 4. One Case.

Mrs. R., aged 35, was first seen on Jan. 16, 1916, by Dr. E. J. Holbrook of San Jose, California, from whom I obtained the following clinical record.

On Friday, Jan. 14, when Mrs. R. opened a jar of home-canned string beans, she noted that the odor was unpleasant. She tasted one of the pods and decided that it was good, but as the beans became heated the odor became much more unpleasant, and, after "nibbling" a pod upon which she had put some salt, she decided that they were not good, and threw them out.

On the following day she did not feel well; there was some dizziness and headache, and she "felt weak." On Sunday she was nauseated and so weak that she was unable to get up; and during the day the weakness became more marked. There was a full movement of the bowels after a course of calomel and magnesium sulfate. During the day a neighbor told her that the chickens which had eaten the beans were all sick, whereupon the patient became alarmed and called Dr. Holbrook.

On examination it was noted that there was bilateral ptosis, double vision, slight difficulty in swallowing, and difficulty in pronouncing certain words. On the following day the ptosis was more marked, speech was more difficult, and the patient was unable to swallow any solid food. On Tuesday she seemed to be somewhat better, but on Wednesday morning there was an attack of extreme respiratory distress which was apparently due to paralysis of the respiratory muscles. Artificial respiration with oxygen was carried on about 2 hours, but the patient failed to rally. Death was due to respiratory failure.

The patient was entirely conscious throughout the illness. There was no pain at any time and no sensory disturbance except a feeling of numbness of the lips. The temperature was never above 99°F., and for a time before death was subnormal. Muscular weakness was extreme, and for the last 24 hours there was apparently paresis of the arms. The urine was normal. On the last day of illness the leukocytes numbered 17,600, of which 86 per cent were polymorphonuclear neutrophils.

Necropsy was performed by me 5 days after death, but, as the body had been embalmed a few hours after death, the tissues were still well preserved. There was very marked congestion of the vessels of the meninges of the brain and lower cord, but no hemorrhage was seen. The tissues of the abdominal and thoracic organs were so discolored by the embalming fluid that it was impossible to determine how much they were congested, but one definite hemorrhage was found in the left adrenal, and one in the wall of the small intestine.

Microscopic examination of the tissues of the brain showed much congestion in the vessels of the meninges on the surface and in the sulci. In some of the veins of the meninges there were thrombi containing bunched leukocytes, of the type which was described by Ophüls, and in several small veins in the tissue of cerebellum and cerebrum there were thrombi in which were fewer leukocytes (Fig. 3). Careful examination of the ganglion cells of the stem, especially in the region of the various motor nuclei, failed to reveal any definite disintegration of the Nissl granules or of the cell protoplasm. Sections of the cord showed nothing abnormal.

Sections of the lungs showed moderate hyperemia and edema, and several

large areas in which the air spaces were filled with blood. The bronchi were filled with purulent material and there were small areas of bronchopneumonia. Several arteries and veins contained thrombus in which were masses of conglutinated blood platelets and many leukocytes (Fig. 4).

The kidneys showed moderate parenchymatous degeneration, exudate in the glomerular capsules, and thrombi containing leukocytes in some of the arteries and veins. Sections of the liver showed little degeneration of the epithelial cells.

Eight chickens which ate the discarded beans became paralyzed and died within 3 days. Necropsy was performed by me on six. Examination of the brain showed marked congestion of the meninges, especially around the base of the brain and upper part of the cord. There were also large hemorrhages at the base. The tissues were all soft and discolored, but showed evidence of general congestion. Microscopic examination showed much degeneration which was probably postmortem, and thrombi containing conglutinated blood platelets in the vessels of the brain, lungs, liver, and kidneys.

From the contents of the crop or gizzard of three of the chickens an anaerobic, Gram-positive bacillus was recovered, which is morphologically and culturally identical with *Bacillus botulinus*, and which produces a virulent toxin when grown in suitable media.

Outbreak 5. One Case.

On Tuesday, May 23, 1916, Dr. C. Bigelow of San Francisco was called in to see Mr. J. C., who complained that he was "going blind." When first seen the patient was lying on a couch from which he arose with difficulty. There was ptosis of the left eyelid, apparent paralysis of the left sixth cranial nerve and diplopia. He stated that he had felt badly on Saturday and Sunday and was much worse on Monday, and complained of soreness in the back and shoulders which he attributed to the fact that he had slipped while cranking his automobile. The eye symptoms developed rather suddenly on Tuesday.

On the following day the symptoms became more severe. Respiration became difficult, the throat kept filling with thick mucus which he was unable to expectorate, the movements of the tongue became impaired, and articulation was difficult. On Friday, May 26, it was noted that "all the tissues in the throat and pharynx were densely swollen, but there was no deposit on the tonsils" (Dr. F. B. Carpenter). Later in the day the edema had disappeared. Culture in Loeffler's serum showed no Klebs-Loeffler bacilli.

On Saturday, May 27, Dr. Tracy Russell made the following notes:

"The patient was sitting on the edge of the bed and a nurse was attempting to remove glairy mucus from the pharynx. After a few minutes he said, 'I am so tired,' and threw himself back on the bed. He was lethargic but would answer questions. He did not complain of photophobia. There was ptosis of both eyelids, double vision, trismus, contraction of the pharyngeal muscles which prevented him from swallowing, tache cérébrale, generalized small petechiæ, and marked Cheyne-Stokes respiration, the last three deep inspirations being assisted by the patient grasping the head of the bed and using the accessory muscles of respiration."

Examination of the eyes was made by Dr. E. W. Alexander, whose report states:

"Subjectively the patient complained of photophobia and double vision. The fields of vision were normal (the perimeter was not used), the central color vision was normal, and there was no tenderness on pressing the eye back into the orbit or pain on rotation (no retrobulbar neuritis). He could see a small test-color object clearly enough to distinguish its color (1 mm. in diameter at 18 inches), hence the intraocular muscles were normal. The pupils were normal in size, shape, and reaction. There was some paresis of the horizontal external muscles, and ptosis of the upper eyelids. The corneal reflex was hyposensitive and the fundi were normal except for slight venous retinal enlargement, which was not more than might occur physiologically."

There was no gastrointestinal disturbance, no headache, no pain except in the back and shoulder, and no disturbance of mentality. There was some dizziness and slight ataxia, and the knee-jerks were somewhat overactive, especially on the left side. The diplopia was transitory. The pulse varied from 80 to 100 per minute, and the respirations from 20 to 30. The urine was clear until the last 2 days when there was a heavy cloud of albumin and the sediment contained a few hyaline and epithelial casts and some red blood corpuscles. The leukocytes varied from 10,000 to 14,000. The Wassermann reaction and the tuberculin test were negative. Examination of the cerebrospinal fluid showed 80 cells per c.mm.

Death occurred on Saturday evening, 4 days after the onset of the eye symptoms. The cause of the poisoning was not determined.

Partial necropsy was permitted; the brain was removed, but the abdominal and thoracic cavities were not opened. Examination of the brain was made by Dr. W. Ophüls whose report is as follows:

"The pia mater and the substance of the entire brain were intensely congested. Fairly large groups of Pacchionian granulations were on the convexity of the brain. Frontal sections were made throughout the cerebrum, pons, and medulla, and, except for general intense congestion, no gross lesions were made out.

"Sections of the brain from various places show very marked dilatation and hyperemia of all blood vessels. There are quite a few microscopical hemorrhages in the pia mater. Several blood vessels (small arteries and veins) in the pia mater as well as in the brain substance are either partly or completely filled with thrombi consisting of hyaline masses (probably conglutinated blood platelets), some fibrin, and many leukocytes. The latter

are mostly polymorphonuclears. Few small similar accumulations of leukocytes occur in the pia.

"An especially careful microscopical study was made of the region of the pons and medulla oblongata. The tissues are not well enough preserved to show the finer details of the ganglion cells, but otherwise the nuclei of the cranial nerves appear normal, except for the congestion and occasional microscopic hemorrhages."

Outbreak 6. One Case.

On July 1, 1916, Mrs. W. of San Pasquale, near Escondido, California, noticed that one of a number of jars of string beans, which she had canned 2 weeks before, was leaking. She tasted one of the pods and noted that it was "slightly sharp," and when she began to "heat them over" she noticed that there was a "strong, disagreeable odor." The contents of the jar were then thrown out.

On the morning of the following day some of the chickens which had eaten the beans were sick and could not lift their heads. Later in the day several more chickens became affected, and by the next day all the chickens which had eaten the beans were ill. A neighbor who raised large numbers of chickens told Mrs. W. that the chickens had limber-neck. About twenty-five of them died within 3 or 4 days after they had eaten the beans.

On the 3rd day after she had tasted the beans Mrs. W. noticed that she "felt queer," was weak and tired, had double vision, and could not open her eyes. Her mouth and throat were dry, the tongue felt too large for her mouth, and she had difficulty in pronouncing words, and in swallowing. She noted that she was very "unsteady" on her feet, "as if on ship-board," and that when she tried to walk she raised her feet high as if going upstairs. She had great difficulty in combing her hair and in picking up small objects. There was no nausea or vomiting.

The symptoms became more severe for several days, but the patient did not remain in bed, although she was unable to do her work and lay down frequently during the day. At times there was severe pain in the back of the head and neck. She thinks her mentality was below normal and that her memory was poor. She was restless but slept well. She noticed that there was an accumulation of mucus in the throat, but there was never any strangling or disturbance of respiration. She summed up her symptoms in the words, "Everything that I tried to do was a great effort." An optician who examined her eyes told me that there was paralysis of the third nerve on both sides, and that there was no retinal involvement.

About 3 weeks after the onset of the symptoms, Mrs. W. commenced a course of osteopathic treatments and physical culture exercises, and in 2 months was practically well. At the present time (7 months after the beginning of the illness) she states that there is a tendency to basilar headaches and that she becomes hoarse after talking for some time; otherwise there is no apparent ill effect of the poisoning.

Outbreak 7. Seven Cases.

On Monday, January 15, 1917, fifteen persons had dinner in a hotel at Escondido, California. Eight partook of salad which had been prepared from home-canned string beans, and, of the eight, seven developed symptoms which were in every way typical of botulism. Four of the patients died, one was seriously ill, and two were ambulatory cases. I had an opportunity of making a personal investigation of this outbreak and of examining three of the patients. I am much indebted to Dr. B. L. Crise of Escondido, Dr. Edward Fly of National City, and Dr. Robert Smart of Coronado, who placed the records of their cases at my disposal, and who permitted me to examine their patients. Coroner Otto Marsh of San Diego County and Dr. George B. Worthington, autopsy surgeon, also assisted me in every way, and provided me with portions of the organs of one case in which necropsy was performed.

There can be no doubt that the bean salad was responsible for the poisoning, as only one of those who ate salad escaped illness, whereas none who did not eat it showed any symptoms. That the poisonous food was served at the evening meal was proved by the fact that one of the victims was a guest at the hotel for that night only. His companion, who also remained over night, did not eat any of the salad, and escaped illness. Two cans of home-canned string beans were used in the preparation of the salad. As the cook is dead it is impossible to obtain definite information as to whether she noted that the beans were spoiled, but one waitress, who assisted in preparing the salad, said that the contents of one of the cans did not taste quite right; and another waitress noticed that the cook had tasted the beans several times while preparing the salad, although she did not hear her say that anything was wrong with them. The beans were served with mayonnaise on lettuce. They were not cooked after they were removed from the cans.

It is probable that only one of the cans of beans was contaminated, since the severity of the illness of the victims varied so greatly. It seems reasonable to presume that the person who failed to develop symptoms after eating the salad received only beans from the unspoiled can, that those who showed mild symptoms received but little from the

spoiled can, and that those who were seriously ill had eaten larger portions of the beans from the contaminated can.

The clinical picture in all the cases was practically identical, except in severity, and can best be illustrated by a description of two fatal cases in which necropsy was performed, and of one ambulatory case which recovered.

Case 1.—Miss N., waitress, aged 27, consulted Dr. B. L. Crise, Escondido, California, at his office on Wednesday, Jan. 17, 2 days after the meal. She complained of great muscular weakness and said she was "all in." Examination showed no apparent cause for her condition. On Thursday morning Dr. Crise was called to the hotel and found the patient in bed, complaining of headache, pain in "the pit of her stomach," nausea and vomiting, double vision, and difficulty in swallowing and in pronouncing certain words. She was extremely prostrated, orientation was slow, the pulse was 120 and rather weak, and the temperature was 96°F. The pupils were dilated but reacted to light, the tongue was heavily coated although the edge and tip were clean, and the skin was moist. Examination of the heart and lungs showed nothing abnormal, except the heart rate, but the abdomen was somewhat distended and tympanitic. There was no paralysis of the skeletal muscles but there was marked weakness. The patient staggered when she attempted to walk and was unable to pick up small objects, although she could feel them without difficulty. There was marked vertigo whenever she moved her head.

Active purgation was instituted and it was noted that an excessive amount of purgative was necessary before evacuation of the bowels was obtained.

On Friday practically all the symptoms had increased in severity. The patient was anxious and restless although she had slept well. She complained of dryness in the mouth and throat, greater difficulty in talking and swallowing, strangling when she attempted to swallow, and inability to clear the throat of mucus. She was extremely weak. Examination showed a marked pallor of the skin, pulse 120, and temperature about 96°F. There was paralysis of accommodation and double and cloudy vision, although vision with either eye was fairly good. The bowels moved after the administration of Glauber's salts. The superficial reflexes were intact.

On Saturday and Sunday the symptoms were still more severe, prostration was extreme, and the patient was unable to raise the arms and legs. There was greater difficulty in swallowing and talking, and the strangling spells were more frequent. The breath was fetid and there was a peculiar ammoniacal odor from the skin. The pulse was still rapid, and on Sunday the temperature rose to 97.5°F.

On Monday there was complete paralysis of the muscles of deglutition, and speech was almost unintelligible. There was bilateral ptosis of the eyelids and the pupils were widely dilated and reacted sluggishly to light. There was still

complete loss of accommodation. Strangling was most distressing and there was much difficulty in removing the thick mucus from the pharynx. Attempts to swallow were followed by strangling and regurgitation through the nose. The pharyngeal reflex was apparently lost as there was no gagging when the mucus was forcibly wiped out with cotton attached to a hemostat. The knee-jerks were sluggish and sensation was unimpaired. There was incontinence of urine.

On Tuesday the general condition was still worse. The pulse was weaker and more rapid, 140 per minute. Death occurred from respiratory failure, the heart beat persisting for some time after respiration had ceased.

The secretion of urine did not seem to be diminished at any time; it showed a heavy cloud of albumin. Blood pressure was taken several times and was always about 120 mm. of mercury (systolic). There was no terminal rise in temperature which would indicate an insufflation pneumonia.

Necropsy was performed by the Coroner's autopsy surgeon after the body had been embalmed. There was marked congestion of the brain and meninges and of the abdominal and thoracic organs. The spleen was large and apparently had been soft. There was no macroscopic evidence of bronchopneumonia. The tissues were not placed in fixing fluid for several days after death.

Microscopic examination showed marked postmortem change in all the tissues. Sections of the brain showed marked congestion in the meninges and in the tissue, but no thrombi or hemorrhages were seen. The ganglion cells of the basal nuclei showed evidence of degeneration, but there was nothing that was not typical of cells which are undergoing postmortem degeneration.

Sections of the kidneys, liver, spleen, and lungs showed marked congestion. There was marked degeneration of the parenchyma of the liver and kidneys which was at least partially due to postmortem change. The spleen contained several small hemorrhages. No typical thrombi were found in any of the tissues.

Case 2.—Mrs. M. P., aged 48, first complained of symptoms on Tuesday, Jan. 16. She noted a "peculiar sensation about the eyes," and a cloudiness of vision which became progressively worse during the day. On Wednesday she had difficulty in walking, "was walking upstairs all the time," and was very tired. Toward evening she began to have double vision. On Thursday she was very weak and had difficulty in talking and swallowing; she said, "my tongue felt as if it was too large for my mouth and there was a sort of constriction in my throat." On Friday she complained of extreme muscular weakness, frontal headache, diplopia, and photophobia. She was unable to swallow and she talked with the greatest difficulty. On Saturday the symptoms were more severe. She was then seen by Dr. Smart, who gave me the following case record.

The patient was extremely weak and had great difficulty in talking. Her speech was almost unintelligible. There was bilateral ptosis, the pupils were widely dilated and reacted very sluggishly to light, and there was a slight conjunctivitis. The jaws could not be opened widely, the lips and tongue were parched, and the tongue was heavily coated. There was thick, tenacious mucus in the pharynx which the patient was unable to raise. Examination of the chest

revealed nothing abnormal in the lungs. The rate of the heart beat was rapid and the sounds were weak, but there were no cardiac murmurs. Nothing abnormal was found in the abdomen. The knee-jerks and plantar reflexes were normal, mentality was unclouded, and taste, smell, hearing, and the cutaneous and deep sensations were unimpaired. There was no disturbance of respiration and the temperature was subnormal.

On Sunday the patient was much weaker. She complained of difficulty in breathing, and, toward evening, the respiration was of the Cheyne-Stokes type. The pulse was very weak at times but responded temporarily to stimulation. There was an offensive, almost uremic, odor to the breath. The urine was diminished in amount and contained a large amount of albumin. The bowels responded freely to magnesium sulfate enemata.

On Monday morning there was little change in the general condition of the patient, but about 4 o'clock in the afternoon she began to fail rapidly. About 3 hours later there was a sudden attack of cardiac failure from which she did not rally. Mentality was unclouded to within a few minutes before death. A striking feature of the whole clinical picture was the combination of subnormal temperature with a rapid, weak pulse, a combination which persisted to the end (Text-fig. 4).

Necropsy was performed by Dr. George B. Worthington, who reported marked congestion of the brain and meninges, and of the abdominal and thoracic viscera. The spleen was small and contained several small hemorrhages. There was no bronchopneumonia.

Microscopic examination of sections of the brain showed marked hyperemia in the meninges and in the brain tissue, and a few small hemorrhages in the region of the basal ganglia. Many of the vessels of the meninges and of the brain contained masses of thrombus in which were a few leukocytes (Fig. 5). The ganglion cells of the basal nuclei were apparently normal, except for the fact that they were shrunken. There was no evidence of any protoplasmic degeneration or of vacuolization.

Sections of the liver showed moderate congestion. There was very little evidence of parenchymatous degeneration, and none of proliferation of the interstitial tissue.

Sections of the kidneys showed moderate congestion and extreme parenchymatous degeneration. Practically all the cells in the convoluted tubules were degenerated, and many of them were necrotic and desquamated. The epithelium of the straight tubules was also severely damaged, and many of the tubules were filled with necrotic debris. The glomerular tufts were filled with blood but were apparently intact. There was no exudate in the glomerular clefts. There were a few scattered areas of chronic interstitial change. Many of the blood vessels contained masses of thrombus in which were numbers of leukocytes, and some of the medium sized arteries were completely plugged. No hemorrhages were seen.

Sections of the lungs showed moderate congestion. Many of the blood vessels

contained masses of loose fibrin but there were no typical cellular thrombi. There was no evidence of bronchopneumonia.

Sections of the spleen showed very little congestion. Many of the vessels in the trabeculæ and in the pulp were filled with dense masses of fibrin in which were moderate numbers of leukocytes (Fig. 6). There were no hemorrhages.

Case 3.—Mr. C. A. B., of San Diego, a travelling salesman, had taken dinner on Monday night and breakfast on Tuesday morning at the hotel in Escondido. He had eaten heartily of the bean salad and had not noticed any unusual taste or odor. He had been suffering from a severe "cold" for several days and was taking daily doses of magnesium sulfate. He did not notice any symptoms except what he attributed to the "cold" until Wednesday, when he found difficulty in driving his automobile because "things seemed to move in front of me." On Thursday and Friday he had still more difficulty in driving his car, and on Friday he was dizzy at times. About this time his wife observed that "the eye clefts were narrow," presumably ptosis, and that the eyelids were swollen. There had been no special weakness and no gastrointestinal disturbances. He had continued taking magnesium sulfate and the bowels had moved daily.

A "peculiar tight sensation in the throat" and transient attacks of diplopia were noted on Saturday. The tongue "seemed thick and moved slowly" on Sunday, and the patient, who is a member of a church choir, had great difficulty in singing at the Sunday services. He described his difficulty in the words "it felt as though my throat was paralyzed and as though I could not get my breath quickly enough." He was extremely tired Sunday night. On Monday the symptoms were more severe and he was unable to drive his car. On Tuesday he had a "faint spell" when at breakfast, and also during the forenoon. He was unable to cover his regular territory during the week but did a certain amount of work each day.

When seen by me the following Sunday he said that he could not remember any marked change from day to day, although he believed that he was not improving, and that he was weaker and more easily fatigued than earlier in the week. His vision had been impaired, there had been frequent, transient attacks of diplopia, and the ptosis and dilatation of the pupils had persisted. He had suffered from attacks of faintness whenever he overexerted himself, and had noticed that he "stepped higher" than normally and it was an effort for him to talk. He soon became hoarse when he attempted conversation. He had been unable to eat as rapidly as usual because of difficulty in swallowing; it was frequently necessary to take a drink of water before he could swallow solid food. The mouth had been very dry but he had not experienced great thirst. There had been no nausea, vomiting, or constipation, and no abdominal pain. Several times at night he had suffered from choking spells, but they had not troubled him during the day, even when he attempted to swallow. There had been no cough. He had complained a great deal of being cold, even when in a warm room. There had been no disturbance of urination.

Physical Examination.—The patient was well developed and well nourished.

The skin was pale and rather dry. There was no cyanosis. After the exertion of undressing there was considerable dyspnea. The pulse varied from 96 to 120 per minute during the examination. There was moderate bilateral ptosis, the left lid being lower than the right. The pupils were dilated, slightly irregular in outline, and reacted somewhat sluggishly to light (direct and indirect). Accommodation was very slow. There was no nystagmus. The tongue was protruded rather deliberately and was heavily coated. The mouths, lips, and pharynx were dry. The chest movements were equal, and there was no impairment of the lungs. The area of cardiac dullness was not increased, the cardiac impulse was not felt, and the heart sounds were feeble and indistinct, but there were no murmurs. The abdomen was slightly distended and soft but there was no tenderness. The liver, spleen, and kidneys were not felt, but the colon was palpable. There was no edema of the ankles or legs. The pharyngeal, jaw, biceps, triceps, abdominal, cremasteric, patellar, knee, ankle, and plantar reflexes were normal. The Romberg test was negative. There was no definite local muscular weakness and sensation was unimpaired. When dressing, the patient had considerable difficulty in buttoning his clothes.

The systolic blood pressure was 136 mm. of mercury and the diastolic 90 mm. (Mercer).

The red blood cells numbered 5,300,000, and the leukocytes numbered 7,000. The differential count showed polymorphonuclear neutrophils, 49.5 per cent, lymphocytes, 35.5 per cent; large mononuclear cells, 3.5 per cent; eosinophils, 4.5 per cent; basophils, 0.5 per cent; and transitional cells, 6.5 per cent.

The urine contained a moderate amount of albumin.

In addition to the three which have been described, there were four other cases in this outbreak, two of whom died. The cook became ill Tuesday night, complained of severe pains in the head and of difficulty in talking and swallowing, and died Wednesday morning. It was thought at the time that death was due to apoplexy, but, in view of the fact that she had eaten a considerable quantity of the beans, it is probable that she died of a very acute poisoning with the *botulinus* toxin.

The wife of the proprietor of the hotel first noticed symptoms on the Friday following the poisonous meal. She went on a business trip to Los Angeles and had great difficulty in going around the streets because of double vision. While in Los Angeles she consulted an optician who prescribed glasses. When she returned home that evening she was very weak and tired, and immediately went to bed. Her illness was severe and prolonged, and the symptoms were identical with those that have been described. She died on Feb. 10, 26 days after she had eaten the poisonous food. Necropsy was not permitted.

On Jan. 27, the red blood corpuscles numbered 5,200,000, and the leukocytes 12,500. The differential count showed polymorphonuclear neutrophils, 77.5 per cent; lymphocytes, 19.5 per cent; large mononuclears, 2 per cent; eosinophils, 0.5 per cent; and transitionals, 0.5 per cent.

The daughter of the proprietor was able to do the work in the kitchen on Thurs-

day and Friday, although she had some disturbance of vision on Thursday and some difficulty in walking on Friday. On Saturday morning she was able to dress herself and to wait on her mother, but by evening was so weak that she was forced to go to bed. She told me that on Saturday she had had the greatest difficulty in balancing her head when she walked, and that it was often necessary to "steady" her head by supporting her chin with her hand. She was extremely ill for several weeks, her symptoms during the first 11 days being almost as severe as those of her mother. The first sign of improvement appeared on the 12th day when she was free from strangling spells for nearly 24 hours. Recovery was very gradual. 21 days after the poisoning she was able to be propped up in bed, and about 2 weeks later was allowed to sit up in a chair. On Mar. 1, 6 weeks after the poisoning, she was able to walk a few steps. She was able to swallow solid food after the 15th day of her illness.

On Jan. 27, there were 5,400,000 red blood corpuscles and 13,000 leukocytes. The differential count showed 78.5 per cent polymorphonuclear neutrophils, 14.5 per cent lymphocytes, 3.5 per cent large mononuclears, and 3.5 per cent transitional cells.

The seventh patient left for Colorado on Tuesday, the day following that on which the beans were served, and the first symptoms developed while he was en route. They consisted of swelling in the throat, difficulty in swallowing, double and cloudy vision, and disturbance of accommodation. He complained of great muscular weakness, which came on suddenly. There was especially marked weakness of the muscles of the neck which he described in the words "when putting on my shoes I found it very difficult to get my head and neck back to normal position, and in many cases it was necessary for me to push my head up with my hands." He found that the double vision was relieved when he wore amber-colored glasses, but that it returned as soon as he removed them. He was troubled with shortness of breath and rapid heart beat, and had no appetite. There were no other gastrointestinal symptoms. His symptoms all disappeared in a few days, with the exception of the "swelling in the throat" and the muscular weakness. On Mar. 1 he wrote me that he had almost completely recovered, although he had not yet regained his former strength.

The duration of the illness of the fatal cases varied greatly. One patient died within 40 hours from the time she tasted the beans, one died on the 7th day, and another on the 8th day after the poisonous meal. The longest course was that of the wife of the proprietor, who became ill on the 4th day after she had eaten the beans, and died on the 26th day. The immediate cause of death in all the cases was respiratory failure.

An interesting development of the investigation in Escondido was the disclosure of the fact that within the past few years there have been

two instances in which numbers of domestic fowl died of limber-neck after eating spoiled home-canned beans. In one of these instances seventeen chickens, and in the other over fifty chickens and several turkeys died. When considered in connection with the frequent occurrence of limber-neck in the outbreaks of botulism which have been recorded in this report, it seems justifiable to suppose that the beans had been infected with *Bacillus botulinus*. Fortunately no persons had tasted or eaten the beans in these two cases.

SYMPTOMATOLOGY AND COURSE.

The best review of the symptomatology of the earlier cases of botulism is contained in the report of Müller (81), 1869, in which the records of 263 cases are carefully analyzed. The earliest observers had noted that the onset of the paralytic symptoms was frequently preceded by acute gastrointestinal disturbances, and Dann (10), 1828, had suggested a division of the symptoms into two groups, (*a*), the primary "irritative" group, and (*b*) the later "paralytic" group. This division was not generally accepted, however, for, as was pointed out by Schlossberger (13) and Müller (81), the primary irritative group of symptoms was frequently absent.

Van Ermengem (34), 1897, reviewed the previous records of the symptomatology and summarized his conclusions as follows:

"Le syndrome botulinique consiste essentiellement en un ensemble de phénomènes neuroparalytiques: troubles sécrétoires des premières voies et paralysies motrices symetriques, totales ou partielles, dependant très probablement de lésions de la moelle-allongee, du bulbe, principalement des noyaux d'origine de divers nerfs cérébraux, et des cornes antérieures de la moelle épinière.

Il se caractérise:

1. par un arrêt de sécrétion ou une hyper-sécrétion de la salive et des mucosités bucco-pharyngées;
2. par une ophthalmoplégie externe et interne plus ou moins complète (blépharoptose, mydriase, paralysie de l'accommodation, diplopie, strabisme interne);
3. par de la dysphagie, de l'aphonie, de la constipation rebelle, de la rétention des urines;
4. par un affaiblissement genéral de la contractilité de tous les muscles volontaires;
5. par l'absence d'un état fébrile, de troubles de la sensibilité générale et de l'intelligence.
6. A cet ensemble phénoménal s'ajoutent souvent des troubles de la respiration et de la circulation, qui peuvent aboutir à une mort plus ou moins rapide par paralysie bulbaire;
7. Enfin, les manifestations caractéristiques ne surviennent, au plus tôt, que douze à vingt-quatre heures après l'ingestion des aliments. Elles sont souvent précédés de troubles gastro-intestinaux passagers, apparaissent graduellement et persistent pendant des semaines."

The first symptoms usually develop in from 18 to 24 hours after the ingestion of the poisonous food, but rarely they may occur earlier, and not infrequently they may be delayed for 2 or 3 days or even longer. Müller (81) records one case in which the initial symptoms appeared 9 days after the poisonous food was eaten, but he notes that he was unable to confirm the report of Lebert (104) in which it was stated that they might appear as late as 14 days or even several weeks

after the ingestion of the poisonous food. The earliest appearance of symptoms which has been noted in the American literature is recorded by Stiles (98) who noticed weakness and irritation in the throat within 2 hours, and suffered from nausea and vomiting about 4 hours after the meal. In the series described in this report the usual length of time which elapsed before the appearance of symptoms was from 36 hours to 4 days, although in two instances the patients became ill in from 4 to 6 hours, and in one there were no symptoms until the 6th day. In general it may be said that when symptoms appear very early, they are usually of a gastrointestinal type; *viz.*, gastric distress, nausea, and diarrhea.

Botulism differs from the majority of types of food-poisoning in that there are usually no acute gastrointestinal disturbances. Occasionally there may be burning and distress in the stomach, early nausea and vomiting, which may persist for several hours or even a day or two, and diarrhea. The vomitus is frequently yellow and extremely bitter and irritating. There is apparently an early inhibition of the motor function of the stomach; Bürger (17) reported that he had washed from the stomach particles of food which had been eaten 5 days previously, and cases are recorded where portions of the poisonous meal have been found in the stomach at autopsy, several days after the food had been eaten. Constipation is an almost constant manifestation of the condition, and may resist all efforts to induce evacuation of the bowels. It may be present from the outset or may develop after several hours of violent purging. Frequently there is moderate distention of the bowels with gas, but there is rarely any abdominal tenderness or rigidity. Occasionally the patients complain of colic. When evacuation is induced, the stool is frequently scybalous in character. Kerner (7) recorded that the stool was often clay-colored as in biliary obstruction, but this does not seem to be the rule.

Perhaps the earliest symptom of botulism in the majority of cases is a peculiar, indefinite indisposition associated with a feeling of fatigue, sometimes with headache or dizziness, and with definite muscular weakness. Not infrequently the acute gastrointestinal symptoms are lacking and the patient associates his condition with the accompanying constipation, and attempts to relieve his symptoms with laxatives.

Disturbances of vision occur very early, in many cases being the first

definite signs of serious illness which are noted by the patient, and it is noteworthy that a fairly large number of cases of botulism are first seen by ophthalmologists and opticians. There may be initial scintillations and dimness of vision, but changes in the retina are rarely found. Kerner (27) referred to a case in which the dimness of vision progressed to complete blindness; Müller (81) quoted from von Faber (1854) that there were eleven cases in the literature in which amblyopia had progressed to blindness; and Ruge (105) reported one case in which he made a diagnosis of papilloretinitis, but these are the only instances in the available literature in which actual damage of the first cranial nerve is recorded. Practically all the authors agree that the disturbances of vision are entirely dependent upon impairment of the extrinsic and intrinsic muscles of the eye.[4]

There is early involvement of the third cranial nerve in all its functions, and blepharoptosis, mydriasis, loss of reflex to light stimulation, and diplopia are practically constant. Occasionally the pupils are of unequal size, and the contour of one or both may be irregular. There is early loss of ability to accommodate for near vision, and complete loss of accommodation soon follows. It is apparently the impairment of accommodation which is responsible for the cloudy vision, as vision with either eye is usually unimpaired for distant objects. Paralysis of the external rectus muscle occurs earlier and more frequently than paralysis of the superior oblique muscle, but occasionally there is complete fixation of the eyeball in the socket, due to paralysis of all the extrinsic muscles. Nystagmus and vertigo, especially on movement, are not uncommon. Photophobia has been recorded in a few cases, and some authors have noted that the vertigo was less marked when bright light was excluded.

Coincidently with, or closely following the onset of disturbances of vision, the patients usually complain of difficulty in swallowing and talking, and frequently there is a peculiar sensation of constriction of the throat. The tongue appears to be larger than normal and is sluggish in its movements. Cases are recorded in which the patient was

[4] I have consulted with Dr. Hans Barkan and Dr. A. B. McKee of the Ophthalmological Department of the Stanford University Medical School, and they agree that the signs which Ruge has described are evidence of retrobulbar neuritis rather than of papilloretinitis.

unable to lift the tongue over the lower teeth, but usually it can be protruded without deviation, although the movements are slow and are performed with evident effort. The tongue is usually heavily coated on the surface although the edges may be clean. There may be complete paralysis of the pharyngeal muscles and frequently there is loss of the pharyngeal reflex. The mucous membranes of the mouth and pharynx as well as of the nasal passages are dry and may be hyperemic and swollen. Ulcerations and aphthous patches on the pharyngeal mucous membrane have been described, and Niedner (106) reported cases in which there was a typical diphtheritic exudate on the tonsils. It is probable that Niedner's cases were complicated with true diphtheria, as a similar angina developed in one of the attendants who had not eaten any of the poisonous sausage. It is not probable that he had to do with a postdiphtheritic paralysis, as the symptoms of angina and the formation of the exudate occurred comparatively late in the course of an illness which was otherwise typically botulism, and, moreover, did not develop in all of his patients.

The difficulty in pronouncing words is apparently chiefly due to the impaired motility of the tongue, although paralysis of the laryngeal muscles is also an important factor. The voice is low and without normal tone, and later in the course of the illness there may be complete aphonia. At first the speech is slow and there is difficulty in pronouncing certain words, but ultimately the difficulty becomes greatly increased, and attempts at speech produce only an unintelligible mumble. Even in the milder cases the onset of fatigue is extremely evident, and speech becomes slower and more difficult and the voice becomes husky after a comparatively short attempt at conversation. Occasionally there is a distressing croupy cough which is unproductive.

The difficulty in swallowing is largely due to paralysis of the pharyngeal muscles. The patients complain that they cannot force the food from the pharynx into the esophagus, but they say that if they can get it started there is no further difficulty. It is probable that the dryness of the mouth is also an important factor, since in the milder cases the patients note that they are able to swallow solid food if they take a drink of water. Attacks of strangling often occur when the patient attempts to swallow or to free the thick, tenacious mucus from the pharynx. As a rule, strangling occurs only in the more severe cases

and may persist until the patient is extremely exhausted, and not infrequently the attacks are accompanied by a return of the food or fluid through the nose, and by insufflation into the trachea and bronchial tree.

A striking feature of this type of food-poisoning is the extreme progressive muscular weakness. Not infrequently the patient's first complaint is of weakness in the arms and legs, and later it may become so marked that he is unable to raise the arms or legs from the bed. It is especially noticeable that there is difficulty in supporting the weight of the head. One of the patients at Escondido told me that even before she was forced to remain in bed it was necessary for her to "steady" her head by supporting the chin with her hand whenever she attempted to walk, and another said that after he had leaned over to lace his shoes it was necessary to push his head back into position with his hand. Many of the patients complain of inability to masticate, and it has been noted that there is a peculiar dull facial expression which is dependent upon weakness of the facial muscles. Some of the earlier authors noted that their patients were able to sit up in a chair, and even preferred to do so when they were quite unable to walk, and they assumed that the muscles of the back were not involved, but later authors do not confirm this observation. The general muscular weakness is rapidly progressive and in the end may lead to a condition of relaxation of the muscles which closely simulates paralysis.

It is seldom, however, that true paralysis of the skeletal muscles is found. There is almost always paralysis of some or all of the muscles of the eye and those of the pharynx and larynx. Wertheim (107) described a case in which there was complete paralysis of the soft palate, and various authors have noted unilateral or bilateral facial paralysis. In general it appears to be true that there may be true paralysis in muscles which receive their motor nerve supply from the cranial motor nerves, and the loss of reflexes in the eyes and pharynx indicates that it is of the lower neurone type. There is usually a more or less marked incoordination of muscular movement in the arms and legs, so that the patients have difficulty in picking up small objects, and walk with an ataxic gait, but, although a few cases are recorded in which there was an absence of the knee-jerks and the Achilles reflexes, in the great majority of cases the superficial reflexes are all intact, although response to stimulation may be considerably diminished.

In view of the many evidences of involvement of the cranial motor nerves, it is surprising that botulism is characterized by an almost complete absence of sensory disturbances. There may be an initial headache, which occasionally persists, and at times there is early distress or even pain in the region of the stomach, but otherwise it is unusual for the patient to suffer any pain. He usually is apathetic and may be somnolent or apparently semicomatose, though not infrequently there is considerable restlessness and sometimes insomnia, and he may become quite irritable because of the difficulty in expressing himself and of clearing the pharynx of mucus. Occasionally there is some loss of memory and orientation may be slow, but it has been recorded from the earliest times that an outstanding feature of the condition is that mentality remains unimpaired throughout the illness.

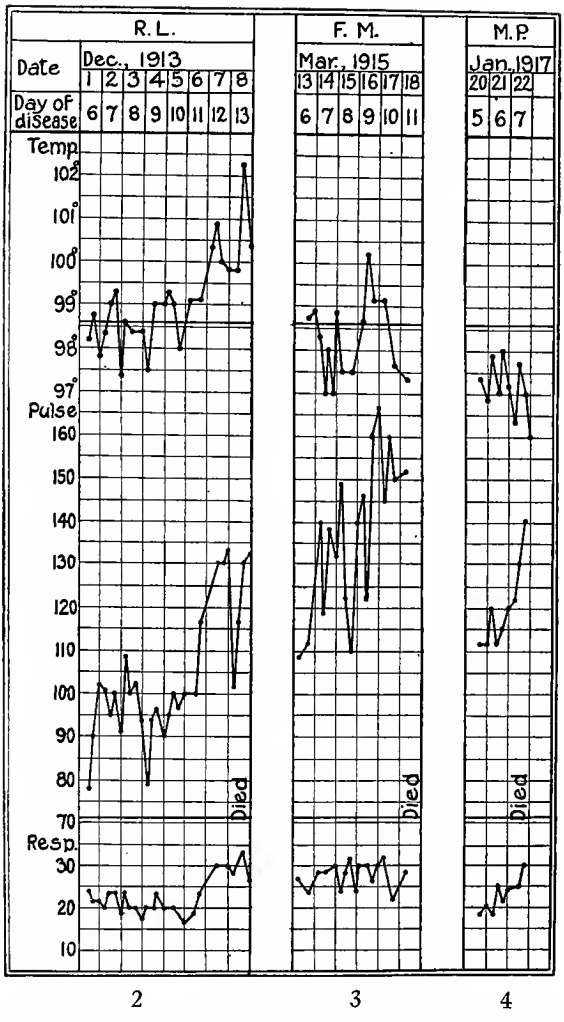
Reference has already been made to the fact that in a very few cases there has been impairment of the optic nerve with blindness. It has also been noted that a few patients complained of tinnitis and partial deafness, but, with these exceptions, there is practically no reference in the literature to any disturbance of the sensory nerves. A few cases were described by the early authors in which the patients complained of loss of sensation in the tips of the fingers and of formication, but Husemann (79) concluded that these were cases in which it was probable that the patients were suffering from trichinosis and not from botulism. Collatz (56) noted that in one of the more chronic cases of his series there were "neuralgic pains" in the neck, and that, associated with paresis of the arms, there was slight disturbance of sensation in the arm and hand. The majority of authors, however, report that their patients were entirely free from sensory disturbances, and this observation has been so frequently confirmed that absence of disturbance of sensation is considered to be one of the characteristic features of the poisoning.

Inhibition of many of the secretions is also an almost constant manifestation of botulism, and the patients usually complain of extreme dryness of the mouth, pharynx, and the nasal passages. Occasionally there is an increase in the amount of saliva, but usually the serous secretions of the mouth are greatly diminished and the mucus is secreted in a thick, tenacious form which is removed with extreme difficulty. It is probable that the absence of serous secretion in the

mouth is largely responsible for the occasional impairment of the sense of taste, although the glossopharyngeal and chorda tympani nerves may also be impaired. A similar inhibition of the serous secretions is found in the gastrointestinal tract and is probably partly responsible for the persistent constipation. Usually there is an inhibition of the sweat secretion and the skin becomes dry and hard. According to Kerner (7), this is especially noticeable on the palms of the hands and soles of the feet where the skin becomes extremely thick and dry and may be desquamated in large patches. Various authors have referred to an absence of tear secretion, and Senckpiehl (15) states that in one case in the literature it was accompanied by severe conjunctivitis. Weiss (9) recorded one case in which the secretion of milk was not diminished and the nursing infant was unaffected, and another in which the patient passed through a normal menstrual period during the course of her illness. There is considerable variation in the reports concerning the secretion of urine. Apparently there is normal secretion in the majority of cases, but instances are recorded in which there was polyuria, oliguria, or even anuria. It is not surprising that the flow of urine should be diminished in the more severe cases, as the patients are unable to swallow any large amount of fluid, although this would be partly counteracted by the inhibition of sweat secretion. It has been variously recorded that the urine is entirely normal and that it contains varying amounts of albumin. Records of renal function tests have not been reported.

The earlier authors emphasized the fact that the pulse rate was extremely slow, and compared it with that which is found in digitalis-poisoning. Senckpiehl (15) noted that at the outset the pulse is frequently 50 to 60 per minute, but that later in the course of the illness it may become as frequent as 100 per minute. A survey of the more recent cases shows that a rapid pulse is extremely common, one of Collatz' (56) cases having a rate as high as 150 per minute. The action of the heart muscle is impaired and an absence of visible cardiac impulse and distant and weak heart sounds are very common.

The temperature is usually subnormal. In the majority of cases it ranges between 96° and 98°F., and in uncomplicated cases it remains subnormal until death. Not infrequently fever develops in the later stages of the illness, but this is usually a sign of the development of



TEXT-FIG. 2. Temperature, pulse, and respiration curves of Miss R. L., the fatal case of the Stanford University series. The patient was not so ill as some of the other patients during the first part of her illness, but developed bronchopneumonia and died on the 13th day. Note the rise in temperature which is coincident with the onset of bronchopneumonia.

TEXT-FIG. 3. Temperature, pulse, and respiration curves of Miss F. M., the elder grandchild in my series No. 1. The patient was taken to the hospital on the 6th day after she had eaten the apricots, and died 5 days later. Note the extremely high pulse and the low temperature.

TEXT-FIG. 4. Temperature, pulse, and respiration curves of Mrs. M. P. Case 2 of Outbreak 7, of my series. Note the rapid pulse rate and the subnormal temperature. The patient was taken to the hospital on the 5th day after she had eaten the bean salad, and died 2 days later.

bronchopneumonia. Eichenberg (108) has stated that fever is never found except in cases which are complicated by pneumonia or some other acute infection.

Respiration is frequently unimpaired in the earlier stages of the illness although it may become accelerated on slight exertion, and may be somewhat irregular. Later in the course of the illness, however, disturbances of respiration become very severe. There may be extreme dyspnea, often irregular breathing, and all the sensory muscles of respiration may be brought into play. Still later there may be partial or complete asphyxia and the most frequent immediate cause of death is respiratory failure. Not only are the accessory muscles of respiration apparently paralyzed, but Fischer (109) records a case in which incision was made to allow of kneading the heart to induce its further action, and the diaphragm was found to be completely paralyzed.

The general appearance of the patient is most distressing. The extreme muscular weakness, the anxiety and the utter helplessness, the difficulty in swallowing, the attacks of strangling, the struggle for breath, and the unsuccessful attempts to articulate constitute a clinical picture which, when once observed, can never be forgotten. The face is usually pale, but in the early stages may be congested. There may be normal appetite and excessive thirst, but the patient is afraid to try to swallow. At times the strangling spells are so severe that there is incontinence of urine, and the accumulation of thick, tenacious mucus in the pharynx is a constant source of annoyance. The fact that the patient remains in full possession of his mental powers and can realize the seriousness of his condition only adds to the distressing character of the situation.

Records of laboratory examinations are very incomplete. In none of the reports in the literature is there any mention of the condition of the blood except that there is a progressive anemia in the more chronic cases. Blood counts were made in five of the cases of my series. In one the leukocytes numbered 7,000 and showed nothing abnormal in the differential count. In two they numbered 12,500 and 13,000, the differential count showing respectively 77.5 and 78.5 per cent polymorphonuclear neutrophils. In one it was noted that the leukocytes varied from 10,000 to 14,000, and in the other that, on the last day of the illness, there were 17,600 leukocytes of which 86 per

cent were polymorphonuclear neutrophils. Subsequent histologic examination showed that in the last case there was a well advanced bronchopneumonia.

A cell count of the cerebrospinal fluid was made in one case and showed 80 cells, but, unfortunately, there is no record of further investigation. The Wassermann reaction in the blood of the same patient was negative. Examination of the urine in several cases showed varying amounts of albumin, but was incomplete in all. Estimation of blood pressure was made in three cases, two of which were very ill, and one which was an ambulatory case, and showed no appreciable variation from normal.

Little can be deduced from the blood counts in these cases as, in all but one, a single examination was made. In the case where two blood counts were recorded there was no opportunity of determining whether the increase of 4,000 cells was dependent upon the beginning of a pneumonic process. In the two cases where there were 12,500 and 13,000 leukocytes respectively, the patients were extremely ill, one subsequently died, but showed no clinical evidence of intercurrent infection. In the case in which the leukocytes numbered 7,000, the patient was an ambulatory case in which the symptoms were comparatively mild.

The duration of the illness varies greatly. Occasionally death may occur within 48 hours after the poisonous food is eaten, but as a rule it occurs considerably later. Müller (81) recorded that of 150 fatal cases, the majority died in from 4 to 8 days after the poisoning, and he added that few persons die who have survived for more than 10 days. In the series of cases which is recorded in this report, one died within 48 hours, one on the 3rd day, seven in from 4 to 6 days, one on the 8th day, one on the 11th day, and one on the 26th day after eating the poisonous food.

Death usually occurs from cardiac or respiratory failure. Frequently it has been recorded that the heart continues to beat for several minutes after respiration has ceased and cases are described in which artificial respiration, especially with oxygen, has kept the patient alive for several hours. Usually there is a terminal asphyxia with its accompanying cyanosis. Not infrequently the patients become comatose some time before death, and in a few cases convulsions

have been noted. Very often they die during an attack of strangling which has been induced by an attempt to swallow food or drink. Occasionally there is apparent improvement in all the symptoms for a few hours or even a day before the sudden failure of respiration or the development of bronchopneumonia precipitates dissolution.

When recovery occurs the convalescence is extremely slow and tedious. The severity of the illness usually reaches its maximum within 10 days and improvement follows slowly. The strangling spells and the difficulty in swallowing and in talking are frequently the first symptoms to diminish in severity. The muscular weakness disappears more slowly. Many cases are recorded in which the patients were comparatively well within 2 or 3 months, but the weakness, vertigo, and emaciation may persist for a much longer time. The disturbances of vision are apparently the last to clear up and may persist for months. The patient frequently retains a tendency to constipation. In so far as can be gathered from the literature there seems to be no permanent disability, although it is frequently several months before the patients regain their former condition of health.

DIAGNOSIS.

There may be difficulty in diagnosis if one has to do with a single case of botulism, especially if there is no available history of the patient's having eaten spoiled food. But when, as occurred in several cases in my series, the illness closely follows the ingestion of food which was known to be spoiled, especially when domestic fowl have shown symptoms of poisoning after eating the discarded food, or when all the members of a family or a number of the guests at a hotel have developed the symptoms within a short time after a meal of which they had all partaken, there is little difficulty in concluding that the illness is due to food-poisoning, and, as a rule, in determining the article of food which is at fault. When one has decided that he is dealing with cases of food-poisoning, the disturbances of vision, the difficulty in swallowing and talking, the absence of sensory disturbances, the marked muscular weakness, and the subnormal temperature at once suggest botulism. Bacteriologic examination of the remnants of the food may reveal the presence of *Bacillus botulinus*, and in the absence of any remnants of food, the recovery of the bacillus from the spleen or intestinal contents of a fatal case, or the demonstration of the typical thrombosis in the blood vessels will establish the diagnosis.

The most common diseases with which botulism may be confused are acute poliomyelitis and cerebrospinal syphilis, but one must also exclude bulbar paralysis, various toxic ophthalmoplegias, and poisoning from belladonna, gelsemium, hyoscyamus, and methyl alcohol.

Lochte (110) and Niedner (106) reported cases in which it was thought that the patients were suffering from hysteria, and it is interesting that in two of the cases of my series the attending physicians at first considered hysteria as a probable diagnosis.

Poisoning from fish roe and from partly decomposed fish which has been salted may produce symptoms that resemble botulism. Novy (111) referred to the so called fugu-poisoning of Japan which is produced by certain fish in which the ovaries and testicles contain a curare-like substance. Certain other fish are poisonous only during

the spawning season, and fish roe-poisoning is not uncommon in Russia. There may be dilatation of the pupils, disturbances of salivary secretion and difficulty in swallowing, but the symptoms are essentially those of a severe gastroenteritis. Konstansoff (112) has shown that the initial products of decomposition of fish are extremely toxic, and that they produce symptoms which resemble those of botulism. The toxin is not destroyed by salting, although the putrefying bacteria are killed, and will produce severe poisoning if the salted fish is not cooked before it is eaten. The poisoning from mussels and oysters may also be of this type, and may closely simulate botulism. Senckpiehl (15) stated that the symptoms of fish-poisoning are usually more acute, are more of a gastrointestinal type, and may be accompanied by a generalized erythema.

MORTALITY OF AMERICAN CASES.

The mortality of the cases which have occurred in this country has been extremely high, but this is probably due to the fact that botulism has not been generally recognized, and that only the fatal cases of food-poisoning have been recorded. Excluding the reported cases in which the diagnosis is not reasonably certain, there have been, during the past 25 years, sixty-four cases of botulism, of which forty-one died, and it is an interesting fact that of the sixty-four cases, fifty-four have occurred in California.

The percentage of fatal cases in the various outbreaks has varied greatly. In Outbreak 1 of this report and in Sheppard's and Frost's series the mortality was 100 per cent, and in Peck's series of twelve cases it was 91.6 per cent. In Wilbur and Ophüls' series of twelve cases, on the other hand, the mortality was only 8.3 per cent, but in the collected cases which have occurred in the United States, the mortality has been slightly more than 64 per cent.

TREATMENT.

The extremely high mortality of botulism is an indication of the unsatisfactory results that are obtained by therapy. The percentage of fatal cases is as great today as it was 50 years ago, and we know very little more concerning treatment than did the physicians of that time. It was hoped that the antitoxic serum would be of value, but there are no available records of its use, except in laboratory experiments. Like the tetanus antitoxin, the *botulinus* antitoxin must be given very early if it is to be effective, and it is probable that, as in tetanus, it is already too late when the symptoms of the poisoning are well established. The fact that Kob (127) demonstrated the toxin in the blood 9 days after the poisoning, indicates, however, that the antitoxin should be given even though the symptoms have been present for some time.

The gratifying results of the prophylactic use of diphtheria and tetanus antitoxins suggest that a similar use of *botulinus* antitoxin may be of value if it is possible to recognize an indication for its administration. The occurrence of limber-neck in domestic fowl, if it develops after they have eaten refuse from the kitchen, should be sufficient reason for administering *botulinus* antitoxin to all persons who have eaten any of the suspected food. Chickens usually develop the symptoms of limber-neck within a few hours, and die within 24 to 36 hours after they have eaten the poisonous food, whereas it is not uncommon to find that human beings are free from symptoms for from 24 to 36 hours after the meal. It is possible that the antitoxin may mitigate the severity of the illness if it is administered before or even shortly after the appearance of symptoms due to disturbances of the central nervous system, and it should be given a trial. Leuchs (114) has shown that polyvalent sera should be used.

Müller (81) outlined the course of treatment which is the basis for all that have since been advocated. Emesis should be induced or lavage performed even when the symptoms have been present for some time, or when the patient has been vomiting, as it is not uncommon to find that portions of the poisonous food are retained in the stomach,

sometimes for several days. Active purgation should be obtained if possible, preferably with magnesium sulfate or castor oil, and the colon should be thoroughly irrigated. The patient should be kept in bed, and as free from excitement as possible. Simple, nourishing food and water should be given in sufficient quantities, but the danger of aspiration pneumonia should be remembered, and it is better to give the water by rectum or by hypodermoclysis when the patient has difficulty in swallowing.

Strychnine seems to be of value in improving the action of the damaged central nervous system and may be given freely. Pilocarpin has been used to counteract the diminished secretions, especially to relieve the distress caused by the accumulation of tenacious mucus in the pharynx, and has given temporary relief in many cases. Pelzl (121) stated that twenty drops of a 1 per cent solution may be given daily without producing toxic symptoms, but Schede (21) reported that in one of his cases, 0.01 gm. of pilocarpin produced a very severe pulmonary edema which almost caused the death of his patient, as he was unable to cough up the fluid. Cardiac and other stimulants should be used as indicated.

Since the illness is due to the action of a poison which is not elaborated within the body, the chances for recovery are good if the patient can be carried over the period in which the toxin is acting. Death is usually due to respiratory failure, and the heart action may be good when the respiratory mechanism is failing. Artificial respiration is therefore indicated, and should be continued, and combined with the administration of oxygen, for hours if the heart continues to beat.

BACILLUS BOTULINUS AND ITS TOXIN.

Van Ermengem (34, 86, 87) was the first to demonstrate the real cause of the poisoning in botulism. From remnants of the poisonous ham, and from the spleen and intestinal contents of a victim in the Ellezelles outbreak, he isolated an anaerobic, spore-bearing bacillus, which produces a virulent toxin when grown under suitable conditions; and he showed that it is the toxin of the bacillus, to which he gave the name *Bacillus botulinus*, which is responsible for the poisoning. In 1906 he obtained another strain from ham which had caused the illness of eight persons in Iseghem in Flanders (87), and his observations have been confirmed by several authors in Europe. In 1900 Römer (35) recovered a strain of *B. botulinus* from ham which had poisoned four persons in Giessen, and in 1901 Madsen (50), in Denmark, obtained a culture from poisonous fish. In 1913 Ornstein (88) found the bacillus in the ham which had caused the illness of two persons, and in the spleen of one of the victims. He recorded that, including his own, four strains of *B. botulinus* had been isolated from poisonous ham, but in 1913 Schumacher (18) recovered another strain from ham which had caused the illness of six persons. There is one record of the isolation of the bacillus from food material which is not of animal origin, that of the Darmstadt outbreak in 1904, in which the bacillus was recovered from home-canned beans which had been served as salad. This strain, which was isolated by Landmann (58), was very similar to that recovered from ham by van Ermengem, although there were certain differences, and various investigators, including Landmann, Gaffky (113), and Leuchs (114), concluded that both were strains of the same organism.

The only record of the isolation of *B. botulinus* from nature is that of Kempner and Pollack (115) who recovered a strain from the intestinal contents of a normal hog. Many materials have been suspected of harboring the bacillus, and van Ermengem (34) made repeated cultures from garden soil, dirt from the streets, mud from ponds and rivers, manure from stables, cow manure, horse manure, duck excreta, and the intestinal contents of several varieties of fish, but in none was he able to demonstrate the presence of *B. botulinus*.

In the course of my investigations of the various outbreaks of botulism on the Pacific Coast, I have succeeded in isolating three strains of *Bacillus botulinus*. The first strain, No. III of my series, was obtained from the crops or gizzards of three chickens which had died after eating the string beans which had caused the poisoning in Outbreak 4; the second strain, No. IV, was found in the gizzard of a chicken which died after eating the discarded corn in Outbreak 2; and the third

strain, No. V, was obtained from a can of string beans which was prepared with that which caused the poisoning in Outbreak 7.

In a review of the available literature I have been unable to find any record that *Bacillus botulinus* has been previously isolated in this country, but I have been informed by Dr. G. L. Hoffmann of the Mulford Biological Laboratories that, several years ago, a strain was recovered from cheese in one of the New England States. Recently a bacillus, which may be *Bacillus botulinus*, was recovered by Dr. Ivan C. Hall, of the University of California, from home-canned corn which had poisoned a number of chickens in Hanford, California, but the identification of this strain is not yet established. Morphologically and culturally it appears to correspond to *Bacillus botulinus*, but, up to this time, I have been unable to demonstrate a toxin production in any of the favorable media.[5]

The method which was adopted in the isolation of the bacilli from the crops and gizzards of the chickens is as follows: The contents of the crop or gizzard were washed into a sterile flask with sterile water, and thoroughly shaken. The flasks were then placed in a water bath at 60°C. and heated for an hour. Films from the contents of the flasks showed a mixture of many kinds of bacteria, including some large, spore-bearing bacilli which had the morphology of *Bacillus subtilis*, and others which were club- or spindle-shaped, also with spores, which resembled *Bacillus botulinus*.

Transplants were made to deep glucose agar cultures which were incubated at 28°C. for several days. All tubes which failed to show gas formation were discarded, but from the others transplants were made to tubes of glucose pork or beef infusion, 0.2 per cent alkaline to phenolphthalein, which were covered with albolene and incubated at 28°C. for about 4 weeks. Films from the agar cultures and from

[5] Since this report was written, Graham, Brueckner, and Pontius have recorded the isolation of two strains of *B. botulinus* from oat hay and ensilage, respectively, which had been responsible for outbreaks of forage-poisoning in horses and mules (Graham, R., Brueckner, A. L., and Pontius, R. L., Studies on forage-poisoning. V. Preliminary report on an anaerobic bacillus of etiological significance, *Kentucky Agric. Exp. Station, Bull. 207*, 1917, 51; Studies on forage-poisoning. VI. An anaerobic organism isolated from ensilage of etiological significance, *Kentucky Agric. Exp. Station, Bull. 208*, 117.)

the infusion cultures showed a mixture of bacteria, among which were spore-bearing bacilli which were morphologically like *Bacillus botulinus*. The odor of the cultures was characteristic. Portions of the unfiltered and also of the filtered infusion produced the typical symptoms and thrombosis in animals.

There was considerable difficulty in obtaining the club-shaped bacilli in pure culture. Repeated attempts at plating were unsuccessful as *Bacillus subtilis* grew so much more rapidly that it overgrew the plates. Strain III was ultimately recovered from the spleen of a rabbit which had received an intravenous injection of the mixture of the bacilli, but Strain IV was only obtained in pure culture after a large series of progressive dilutions had been made in deep glucose agar. In both instances the organism proved to be a virulent strain of *Bacillus botulinus*. Strain V was not contaminated but was obtained in pure culture from the can of beans.

The recorded descriptions of the morphology and the cultural characteristics of *Bacillus botulinus* are based upon a study of the Ellezelles strain (van Ermengem, 34, 86) and of the Darmstadt strain (Landmann, 58). A comparative study of these two strains was recorded by Leuchs (114), and an investigation of these and one other strain was made by von Hibler (116).

The bacilli are large, 4 to 6 by 0.9 to 1.2 microns, have slightly rounded ends, and may arrange themselves in pairs, end to end, or, in unfavorable environment, in long chains. When grown under favorable conditions they form oval spores which are usually situated in the ends of the bacilli, giving them a club-shaped appearance, but they may be placed in the center, producing spindles (Fig. 1). The bacilli are slightly motile and have from four to eight flagella arranged around the periphery. They stain by the Gram method but are apt to be decolorized if left too long in the alcohol.

The organism is a strict anaerobe, although it may grow under imperfect anaerobic conditions if in symbiosis with certain aerobic bacteria, with the white Sarcina (van Ermengem, 87) or with *Bacillus subtilis* (Römer, 35); and, according to Harrass (117) and Tarozzi (118), will grow in freshly prepared bouillon under aerobic conditions if a piece of sterile flesh or potato is placed in the bottom of the culture tube. The addition of glucose to the culture medium greatly increases its activity in growth and in toxin formation.

In glucose gelatin plates, in an oxygen-free atmosphere, the young colonies appear as transparent, pale yellow spherules which are surrounded by a zone of liquefaction. As they grow older the transparency is lost, the colonies become opaque, darker in color, and are nodular in appearance. Gelatin is quickly and completely liquefied and the bacteria collect in flakes at the bottom of the tube, leaving the supernatant fluid clear. In glucose bouillon the medium is at first cloudy but soon becomes clear as the bacteria settle in the bottom. In all media in which there is glucose the bacteria grow more readily than when glucose is lacking, and there is marked and continuous gas formation. According to van Ermengem (34, 86), the bacilli fail to develop normally, and produce involution forms and but little toxin in media which are in every way suitable except for the presence of glucose. He believes that the amount of gas formation is an indication of the activity of the organism, especially in toxin production. The gas contains among others, H_2, CO_2, and CH_4.

In milk, according to van Ermengem (34, 86), there is practically no change in the appearance of the medium, and there is no coagulation, but, according to von Hibler (116), there is precipitation of the casein and peptonization of coagulated protein. The bacilli grow luxuriously in bouillon to which lactose and saccharose have been added, but there is no formation of gas.

The reaction of the medium is of great importance to the growth of the bacillus, especially in so far as the formation of toxin is concerned. Van Ermengem (34, 86) found that the Ellezelles strain would not form toxin in an acid medium, but Leuchs (114) obtained a fairly virulent toxin from the same strain in medium of neutral reaction. The latter author once succeeded in producing a toxin of low virulence by the growth of the Darmstadt strain in acid medium, but, on the whole, the Darmstadt strain required a more alkaline medium for its optimum development than did the Ellezelles strain, and both strains produced much stronger toxin when grown in medium of slightly alkaline reaction.

A small amount of sodium chloride, 0.5 per cent, is necessary for the growth of the bacillus, but too much salt will inhibit its development. Van Ermengem (34, 86) found that 2 per cent sodium chloride was deleterious to the growth of the bacillus in bouillon, but Forssman (119) found that from 0.6 to 1 per cent sodium chloride was

harmful. The growth of the bacillus is completely inhibited in the presence of 6 per cent sodium chloride, a fact that is of considerable economic importance, as meats which are pickled in brine which contains more than 6 per cent sodium chloride will not become contaminated with the toxin.

Van Ermengem (87) obtained the strongest toxin when he grew *B. botulinus* in a medium consisting of beef infusion to which he added 1 per cent sodium chloride, 1 per cent peptone, and 2 per cent glucose, but Leuchs (114) preferred a medium prepared from infusion of pork (500 gm. per liter) to which was added 0.5 per cent sodium chloride, 1 per cent glucose, and 1 per cent peptone. He added calcium carbonate to each container. Van Ermengem (87) recorded that the organism will not grow on peptone-free glucose media such as were used by various authors for studying the fermentation of the Klebs-Loeffler and tubercle bacilli, and Landmann (58) stated that it is impossible to produce the toxin unless animal protein is present.

The optimum temperature for the growth of the bacillus and for the development of the toxin lies between 20° and 30°C. Van Ermengem (34) found that the Ellezelles strain grew very slowly below 16°C., but that its growth was fairly profuse between 18° and 20°C. At from 37° to 40°C. the colonies appeared early, but there was little gas formation, and after about 48 hours the development ceased. At this high temperature there was never any spore formation, the bacilli grew in long chains, and soon developed involution forms. There was never any toxin. Landmann (58) found that the optimum temperature for the Darmstadt strain was about 24°C., but he observed spore formation at 36°C. and obtained a toxin of low virulence at 37.5°C.

Spore formation occurs early, within 48 hours, when the organism is grown upon suitable medium at the optimum temperature, but is delayed or even prevented if the medium or the temperature is unfavorable. According to Van Ermengem (87), the spores are considerably less resistant than are those of the other pathogenic anaerobic bacilli; they are destroyed by heating at 80°C. for $\frac{1}{2}$ hour, at 85°C. for 15 minutes, or by boiling for 5 minutes. They remain viable for a long time, for months or even a year, if protected from the action of light and air, even though the medium in which they are immersed is of acid reaction.

The bacilli are apt to die unless they are transplanted from time to time. Van Ermengem (34) found that if subcultures were made every 4 to 6 weeks, the virulence was maintained, but Forssman (119) found it necessary to return the bacilli frequently to the glucose pork infusion medium. In laboratory cultures there is a tendency for the bacilli to lose their power of splitting glucose with the formation of gas, and of producing toxin, and, in so far as I have been able to learn, there is no reliable method of restoring these properties when once they have been lost.

According to van Ermengem (34), *B. botulinus* is strictly saprophytic and will not reproduce or produce its toxin in the animal body. When washed bacilli are injected subcutaneously into animals, no symptoms of botulism develop and the bacilli rapidly disappear from the site of injection. After intravenous injection of bacilli, cultures of the spleen and liver may show that they are present in these tissues, but they are so few in number that they cannot be demonstrated in microscopic sections. Guinea pigs and rabbits can take large numbers of bacilli by mouth without showing any signs of poisoning, and large numbers of spores may be fed or injected without producing symptoms. Van Ermengem (34) inoculated glucose agar blocks with spores of the virulent strains, and placed them in the peritoneal cavity of a guinea pig, and he also duplicated Vaillard and Rouget's method (120) of intensifying tetanus intoxication by injecting the washed spores under the skin with lactose and sodium carbonate, or with other forms of bacteria such as *B. prodigiosus*, without obtaining any evidence of poisoning. He concluded that the organism could not form its toxin in the bodies of warm blooded animals, probably on account of the high body temperature.

The cultural characteristics of the strains which I have isolated correspond in most respects with those that have been detailed. Strains III and IV produce a highly virulent toxin when grown on glucose beef or glucose pork infusions, prepared according to the formulæ of van Ermengem (86) and Leuchs (114) respectively; 0.0001 cc. of the toxin of Strain III and 0.0002 cc. of the toxin of Strain IV will kill a guinea pig within 24 hours. Sufficient toxin is formed within 4 days (Strain III) to cause the death of a guinea pig 24 hours after 1 cc. is injected subcutaneously.

Cultures were made in several of the sugars and showed similar results with Strains I, II, III, and IV. Strain V has not been tested. When grown in Dunham's solution containing the sugars, there was acid and gas formation with glucose, maltose, levulose, and galactose.

In the dextrin-Dunham solution there was acid formation but no gas, and with lactose, mannite, inulin, and saccharose there was neither acid nor gas. A series was grown in the Hiss serum water containing sugars, but in these the reactions were very irregular. The medium was acidified, coagulated, and ultimately peptonized in practically all the tubes, and there were varying amounts of gas in all, but in view of the results with Dunham's solution it is probable that the gas formation depended upon the splitting of the proteins.

The reactions of all four strains upon litmus milk are interesting in view of the fact that the earlier authors had found no change. In all the tubes there was precipitation of a finely divided coagulum, without the formation of acid, and, eventually, complete peptonization. The precipitation began in Strain III on the 2nd day, but in the others was first noticed in from 4 to 6 days. Peptonization began shortly after the precipitation, and was not complete for from 10 to 12 days. The reaction is probably the same as was described by von Hibler (116), and is of especial interest in that the two virulent strains produced the same reactions as the two which are of low virulence. Van Ermengem (87) believed that von Hibler's results had been due to the fact that his strains had deteriorated, as their virulence was very low, but Strain III, the most virulent of my series, produced the reactions more quickly than did either of the old and relatively non-virulent strains, I and II. The peptonization of the coagulated protein corresponds to observations of von Hibler, but he did not record that the bacilli will also coagulate the protein.

The toxin of *Bacillus botulinus* is formed when the organism is grown upon suitable media under strictly anaerobic conditions. It may develop in small quantities at temperatures below 20°C. and above 30°C., but at the ordinary body temperature of warm blooded animals it is rarely produced. It is analogous in many respects to the toxins of the Klebs-Loeffler and the tetanus bacilli, but differs in that it is not destroyed by the action of the gastric secretions.

Van Ermengem (34) showed that it is an albuminous substance which is precipitated by alcohol, tannin, and neutral salts, and Brieger and Kempner (122) obtained it in a dried form by precipitation with zinc chloride. Their method was as follows: One part of the filtered bouillon culture was heated with two parts of 3 per cent zinc chloride after traces of ammonium hydrate had been added if

the medium was acid. The precipitate was separated and washed, and the solution was made slightly alkaline with 1 per cent ammonium carbonate. The zinc was precipitated with ammonium phosphate, and filtered off. The toxin was then separated from the albumoses by the addition of ammonium sulfate. At this stage the toxin was practically full strength, but if further purification was attempted, it rapidly lost from 40 to 50 per cent of its virulence. The authors found that the dried toxin was resistent to the action of reducing substances but that it was highly susceptible to alcohol, ether, and oxidizing reagents. If protected from light and air it would retain its full virulence for several weeks, and a diminished virulence for more than a year.

The toxin in solution is quickly destroyed by exposure to the action of light and air, but will maintain its virulence for 6 months or more if kept in the dark, and sealed. According to van Ermengem (34), the toxicity is diminished by heating at 56°C. for 3 hours, and is destroyed by heating at 80°C. for half an hour or by boiling. The toxin dialyzes slowly, and is not affected by drying. It is insoluble in alcohol, ether, and chloroform. The addition of 20 per cent by volume, of normal soda solution destroys it in a short time, but an equal amount of normal hydrochloric acid does not reduce its virulence in 24 hours. Putrefaction has no effect on the virulence of the toxin if the access of air is prevented, and the growth of other saprophytic bacteria in the medium does not injure it.

The virulence of the toxin varies greatly, depending upon the strain with which the culture medium is inoculated, the medium upon which it is grown, and the conditions of anaerobiosis, temperature, etc., in which it has developed. In laboratory experiments it has been possible to obtain a toxin of which 0.000001 cc. would kill a 250 gm. guinea pig in from 3 to 4 days (Brieger and Kempner, 122). In the Ellezelles outbreak (van Ermengem, 34) about 200 gm. of the poisonous ham were sufficient to cause the death of one of the patients; in the Darmstadt outbreak (Fischer, 57) two spoonfuls of salad were fatal, and in Quincke's series, a piece of preserved duck, the size of a walnut, was sufficient to cause an illness which lasted for 8 weeks. In my series of cases, one patient died after tasting a small spoonful of the spoiled corn, one died after "nibbling" a portion of a pod of the spoiled string beans, and a third was ill after "tasting" a pod of beans which she did not swallow.

The culture medium which has been used by almost all investi-

gators in preparing toxin has been made from beef, pork, or mutton, but Leuchs (114) reported that Gaffky had told him that he had succeeded in producing the toxin in a medium prepared from white beans. In my series of experiments the strongest toxin was produced in pork and beef infusion, but virulent toxins were also produced in media prepared from string beans, green peas, and green corn, respectively; and much less virulent toxins were obtained in media prepared from asparagus, artichokes, peaches, apricots, and crushed apricot stones.

The method in which the toxin acts upon the tissues is unknown. There is comparatively little local reaction at the site of subcutaneous injections in animals, and there are no characteristic local lesions in the stomach when the toxin is administered by mouth. Kempner and Schepilewsky (123) showed that there is an especial affinity for the tissues of the central nervous system as in tetanus. They found that 1 cc. of an emulsion of the brain or cord of a guinea pig will neutralize from three to four times the lethal dose of the *botulinus* toxin and that there is a partial protection if the toxin and the emulsion are simultaneously injected on opposite sides of the body of a white mouse. They also found that there is a delay in the onset of the symptoms if the tissue emulsion is injected 6 hours before the toxin is administered, and that the animals live for about 1 day longer than the controls; but when the emulsion is injected 12 hours before the toxin, the animals die as soon as the controls. They were unable to increase the protection to more than four times the lethal dose by mixing with larger amounts of the emulsion. Cholesterol and lecithin protect as well as the brain tissue emulsion, and butter and emulsified oil neutralize about twice the lethal dose. Tyrosine and antipyrine also protect to a certain extent. Emulsions of other tissues have no effect.

Van Ermengem (34, 86) investigated the effect of the toxin when administered by mouth, subcutaneously, intraperitoneally, intravenously, and intraocularly. He found that the symptoms appear earliest after intravenous injection, and that they appear somewhat later, but at about the same time, after subcutaneous and intraperitoneal injections. Guinea pigs and monkeys are susceptible to poisoning when the toxin is administered by mouth, although a larger amount of toxin is required; but rabbits and cats are much more resistent to the administration by mouth, although they are susceptible to subcutaneous, intraperitoneal, and

intravenous injections. Rabbits are not affected when the toxin is dropped into the eyes, but when it is injected into the anterior chamber of one eye, symptoms of involvement of the third nerve appear simultaneously in both eyes.

Lippmann (124) estimated the difference in the amount of toxin necessary to poison a white mouse when it is administered by mouth, and subcutaneously, and found that of a toxin in which the lethal dose by subcutaneous injection was 0.000025 cc., the amount necessary to give by mouth was 0.04 cc. Forssman (125) found that if the toxin is placed in the large intestine there are no symptoms of poisoning, even though from 1,000 to 2,000 times the lethal subcutaneous dose is injected; and in test-tube experiments he showed that 10 gm. of the cecal contents of a rabbit will precipitate 1,000 times the lethal dose of toxin, although the precipitation does not destroy the virulence of the toxin. The contents of the small intestine are less active than those of the cecum, 10 gm. of the contents of the small intestine will precipitate about 90 times the lethal dose of toxin. Forssman concluded that the larger dose which is required to produce poisoning from the gastrointestinal tract is dependent upon this precipitation of the toxin.

Forssman (125) also investigated the effect of direct administration of the toxin into the brain by using the methods of Roux and Borrel (126) with which they demonstrated that there is a peculiar virulent type of "cerebral" tetanus. He found that there is no "cerebral" botulism which differs from that produced by other methods of injection, a fact which is somewhat surprising in view of the neutralizing effect of the tissue of the central nervous system which was demonstrated by Kempner and Schepilewsky (123).

The only evidence that there may be some local effect of the toxin of *B. botulinus* is given by Forssman (125) and Leuchs (114). Forssman found that if the toxin is injected into the peritoneal or pleural spaces, the respiratory symptoms appear much earlier than when it is administered by other routes, and he believed that this is due to the fact that there is a local effect upon the diaphragm which causes it to become paralyzed earlier. Leuchs injected the toxin into one of the extremities and observed a condition analogous to local tetanus, in which the symptoms appear first in the extremity into which the toxin has been injected. Neither author suggested an explanation for the phenomenon.

The fate of the toxin after it enters the blood stream is also unknown. Kempner and Schepilewsky (123) found that the brain of a guinea pig which had died from botulism was non-toxic, and other authors have recorded that the tissues of animals which had died after injection showed no trace of the toxin. The only record of the identification of the toxin after it has entered the blood stream is reported by Kob (127), who found that blood serum taken from a patient 9 days after she had eaten the poisonous food, and 3 days after the appearance of the paralytic symptoms, produced the typical symptoms when injected into white mice.

Van Ermengem (34, 86, 87) investigated the effect of the *botulinus* toxin on various kinds of animals. He found that white mice, guinea pigs, rabbits, cats, pigeons, and monkeys are susceptible to subcutaneous, intraperitoneal, and intra-

venous injection, and that white rats, dogs, chickens, frogs, and fish are highly resistent. In his feeding experiments he found that mice, guinea pigs, and monkeys are especially susceptible, that rabbits are less susceptible and that cats must be given enormous quantities of the toxin before they show any symptoms. He found that dogs, rats, and chickens are practically unaffected, the only result of very large doses being vomiting, diarrhea, and emaciation.

It is surprising that van Ermengem obtained no positive results in his experiments with dogs and chickens, especially with chickens, as in the series of outbreaks of botulism which I have investigated it has been the rule to find that varying numbers of chickens have died from limber-neck, a disease in which the clinical picture is entirely analogous to that which occurs in botulism in susceptible animals; and in one case a dog, which had eaten the discarded food, became paralyzed in the hind legs. In one instance, a number of turkeys were also ill with limber-neck, and in one it was proved that the limber-neck was caused by the discarded food, as all the chickens in the pen into which the food was thrown became ill and died, whereas none in the adjoining cages was affected. In my laboratory experiments I have found that chickens are highly susceptible to subcutaneous injection and to feeding, and that dogs are also susceptible to subcutaneous injection. In one of my experiments a 14 pound dog died within 3 days after a subcutaneous injection of 1 cc. of a pork infusion culture; the amount of toxin per kilo being about 600 times that necessary to kill a guinea pig in the same time. In a series of five experiments with dogs, four died and one recovered, and the symptoms were practically identical with those that are observed in cats. Similar results were obtained with toxin prepared from Strains III and IV of *Bacillus botulinus*.

The symptoms which are produced in the different animals are similar to those which are observed in human beings, although they vary somewhat in the different species. There are disturbances of vision, the pupils are dilated, reaction to light is impaired, in monkeys there is ptosis, and in rabbits, guinea pigs, and chickens there is sluggish movement of the nictitating membrane. There may be prolapse of the tongue, an increase or a decrease in salivary secretion, and marked constipation. The animals are weak, and there may be local or general paresis of the skeletal muscles. A characteristic

attitude is that the animal lies on its belly on the floor with the head resting on one side and the legs sprawled out on either side. Respiration may be rapid but is sometimes labored and slow, and death may be preceded by convulsions. In cats there is thick mucus in the pharynx, and the animal has a persistent toneless cough.

When a sublethal dose of the toxin is given, the animals may be very ill for a time and then gradually recover. Often, however, they become emaciated and cachectic and may die from exhaustion after several weeks.

The pathological anatomy is discussed in another section of this report.

Kempner (128) was the first investigator to study the immunology of botulism. In his first experiments he was unable to demonstrate any antitoxin formation in small animals, as he was unable to keep them alive after the initial inoculation. In 1897, however, he recorded the successful immunization of goats, in which he obtained a serum of such potency that it would neutralize ten times the lethal test dose for a guinea pig. He found that the antitoxin would protect an animal against an injection of toxin given 30 hours after the antitoxin, and that if the antitoxin was given 24 hours after a 48 hour fatal dose of the toxin it would save the life of the animal in many cases.

Forssman and Lundstrom (129), 1902, succeeded in immunizing smaller animals by attenuating the initial dose by heat, and were able to obtain a high degree of immunization in goats in a much shorter time than Kempner (128). They confirmed Kempner's observations regarding the prophylactic powers of the antitoxin, but were unable to demonstrate any protection when the antitoxin was given more than 12 hours after the toxin. Forssman and Lundstrom agreed with Kempner that if the antitoxin is to be of any value as a therapeutic agent, it must be given very early.

In a later report Forssman (130), 1905, stated that it is impossible to determine any relation between the amount of antitoxin that is formed, and the amount of toxin injected. He found that after subcutaneous injection of the toxin there is much more antitoxin formed than after an equal amount injected intravenously, and that the maximum of antitoxin formation is reached in 15 days in the former, whereas it required only 10 days in the latter.

Leuchs (114), 1910, performed a series of comparative investigations with the antitoxins of the Ellezelles and the Darmstadt strains, which he obtained by immunizing horses, and found that each antitoxin was specific for the homologous toxin, but had no protective action against the other. He concluded that for therapeutic purposes it is necessary to have a polyvalent antitoxin. Leuchs also showed that an animal could be protected against subcutaneous injection of toxin if the antitoxin is given by mouth, but noted that if the toxin-antitoxin

mixture is given by mouth, an excess of antitoxin is required, as a unit of antitoxin given by mouth will not counteract a corresponding amount of toxin in the stomach. He referred to some experiments which showed that the virulence of the toxin is increased if it is mixed with horse serum, and that the alcohol-soluble portion of the serum, as well as an emulsion of lecithin, will increase the virulence if added in equal volume to the toxin.

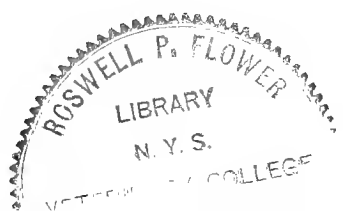
Tchitchkine (131), 1905, attempted to immunize rabbits by giving the toxin by mouth, but was only partially successful, and Lippmann (124) in 1910 showed that while the feeding of toxin by mouth did not produce immunity to subcutaneous injections, it produced a local immunity to the extent that the animal would survive ten times the fatal dose of toxin by mouth.

Serological investigations have not shown very definite results. Herman (132), 1900, reported that he had found that the serum of animals with botulism would agglutinate the bacilli in dilutions of 1:400, and Leuchs (133) recorded that there is weak agglutinating power. The latter author also stated that there is a faint complement fixation reaction, but added that as the experiments were performed with unfiltered bouillon, there is some doubt as to the accuracy of the results.

PATHOLOGY.

The earlier descriptions of the pathological anatomy of botulism need only be mentioned. Kerner (7) noted that there was marked congestion of all organs, and emphasized the fact that there was very early and persistent rigor mortis, and that putrefaction occurred very late. Various other authors agreed as to the general hyperemia but differed greatly in their descriptions of the other changes. Hasselt (134) wrote: "Die angegebenen Leichenerscheinungen sind überhaupt nicht constant, wie man auch von einigen Autoren mehr Entzündungserscheinungen, von Anderen mehr septische hervorgehoben findet." Müller (81), 1869, collected all the available necropsy findings and carefully analyzed them to determine what pathological changes were most constant. He concluded that the great differences in interpretation among the earlier authors were due to the fact that they did not recognize the difference between true inflammation and hypostasis; and that many of the lesions which had been described were merely manifestations of postmortem change. His analysis showed that there was no inflammatory process, and that the only characteristic feature was a general hyperemia. He did not find any conclusive evidence of the rapid development of rigor mortis, or of delayed putrefaction.

The first important investigation of recent times was recorded by van Ermengem (34, 86, 87), whose report dealt with the changes which were found in animals in which botulism had been experimentally produced. He noted that the macroscopic appearance of the tissues in animals was the same as in those of the persons who died in the Ellezelles outbreak, but he was unable to compare the microscopic pathology as the examination of the Ellezelles cases was incomplete. He described the general appearance of the tissues in botulism in the words: "Elles (les lésions macroscopiques) consistent surtout. . . . en un état hyperémique plus ou moins prononcé de tous les organes s'accompagnant souvent de ruptures vasculaires, d'extravasations sanguines" (34). At the site of subcutaneous injection there was slight injection and local edema in the more susceptible animals, and marked local reaction, sometimes with pus formation, in the more resistant; namely, cats and dogs. Local necrosis was never found. When the toxin was administered by mouth there were various changes in the mucosa of the stomach which van Ermengem believed to be due to the local action of the toxin. The author ascribed especial importance to the enlargement and parenchymatous degeneration of the liver, although he noted that the kidneys were similarly involved. In a number of cases in animals as well as in the victims of the Ellezelles outbreak, he found areas of bronchopneumonia in the lungs. The brains and cords were soft, more so than he believed could be due to postmortem change, and contained numerous small hemorrhages.



Microscopic examination of the tissues of the animals was made under the direction of van der Stricht (135), and a special study of the central nervous system was conducted by Marinesco (136). The tissues were fixed by various methods, and special tissue and bacterial staining methods were used.

Van der Stricht (135) recorded changes in the stomach, small intestine, liver, kidneys, spleen, heart muscle, lungs, bone marrow, and submaxillary glands. In the stomach there was hyperemia, usually associated with hemorrhages in the mucosa and ulcerations. There was desquamation of cells in the region of the orifices of the glands, and fatty degeneration of the cells of the glands. In the small intestine there were more or less marked evidences of enteritis in addition to the hyperemia and hemorrhages. The liver was markedly congested and contained small hemorrhages. There were evidences of inflammation of the interstitial connective tissue as well as marked parenchymatous degeneration of the epithelial cells and fatty degeneration of the endothelial cells of the capillaries. The kidneys were markedly congested and there were parenchymatous degeneration and desquamation of the epithelial cells. Degeneration of the endothelial cells of the glomerular tufts was not noted. In the region of the papillæ there were areas of interstitial proliferation. The spleen was sometimes smaller, but more often larger than normal; when larger, the increase in size was apparently due to engorgement of the splenic pulp. There were many large phagocytic cells in the vessels of the pulp. The lungs were congested and sometimes showed areas of peribronchial pneumonia. Hemorrhages were also described. There was proliferation of many of the cells in the bone marrow. The submaxillary glands showed fatty degeneration of the mucous cells. The muscle fibers of the heart muscle and of skeletal muscles showed fatty degeneration.

The changes which were found in the central nervous system are of especial interest in that they seemed to give an anatomical explanation for the peculiar combination of symptoms in botulism. The following description of these changes is quoted from van Ermengem (86):

"Beim Centralnervensystem sind die Läsionen in dem Grosshirn fast Null; sie fehlen in den Nervenfasern und sind sehr ausgesprochen im Rückenmark, weniger in der Medulla oblongata, Protuberanz und Gehirnschenkel. Im Rückenmark und Pons sind die fast ausschlieslich in dem Vorderhörner und Hinterhörner (motorische Kerne der Glieder und Bulbärkerne) vorhanden. Im ersten Stadium findet man nur in den Nervenzellen eine von der Peripherie ausgehende Verminderung der chromatophilen Elemente, später sind die Nissl'schen Körperchen in feine pulverartige Granula umgewandelt. Noch später entstehen Vacuolen durch Auflösung der achromatischen Substanz. Diese chromatolytischen Degenerationserscheinungen sind im Kern der N. hypoglossus, im Nucleus ambiguus, im Nucleus dorsalis des N. vagus, in den Purkinje'schen Zellen des Kleinhirns und im Mittelkern des Oculo-motorius beobachtet worden. Neben diesen regressiven Läsionen der Nervenzellen bestehen stets progressive Veränderungen des Gliagewebes. Die Gliazellen sind an mehreren Stellen vermehrt und spielen die Rolle von Neurophagen, indem sie die krankhaften Elemente wegschaffen. Endlich, seitens der Blutcapillaren treten

auch constant hämorrhagische Herde, mehr oder minder diffus, interstitiell in der grauen Substanz der Hinterhörner, hauptsächlich der Bulbär und der Oculomotoriuskerne auf."

The observations of Marinesco (136) have been confirmed by several authors. Kempner and Pollack (115) agreed with Marinesco in as far as the distribution and type of lesions in the nerve cells are concerned, but found an earlier stage of protoplasmic degeneration which they described as a "*klumpige Schwellung*" of the cell granules. They did not find any evidence of proliferation of the neuroglia cells. They noted that in cases in which a full lethal dose was given (an amount sufficient to kill the animal in 48 hours) and followed by antitoxin within 24 hours, the life of the animal would be saved; but the lesions in the nerve cells were still present 2 weeks later, and apparently disappeared very slowly. They stated that the cells were evidently damaged for a long time after the disappearance of the clinical symptoms, and that they could not find any correspondence between the severity of the lesions and that of the clinical symptoms.

Ossipoff (137) made a study of the changes produced in guinea pigs, cats, and monkeys, and also found the lesions which had been described by Marinesco. He noted that they were most severe in the cord, especially in the portions which supply the nerves for the extremities, and that they were less numerous in the bulb, but he also described changes in the Purkinje cells of the whole brain, whereas Marinesco has stated that none were to be found in the cells of the brain. An interesting feature of Ossipoff's report is his observation concerning the blood vessels. He wrote: "Dans les vaisseaux hyperémiés ainsi qu'en dehors d'eux on rencontrait aussi beaucoup de globules blancs. Dans les vaisseaux il y avait parfois une agglomération de ces globules." He concluded his report by stating that he had not found sufficient reason to assume that the changes which are produced in the nerve cells are specific of botulism, and different from changes produced by all other toxins such as tetanus or diphtheria, but he believed that it is probable that the cell changes in botulism are more intense and more extensive.

Römer and Stein (138) made a careful investigation of the region of the nuclei of the third cranial nerve in monkeys which had been poisoned with *botulinus* toxin. They observed extensive changes which were especially marked in the cells of the unpaired median nucleus. The cells showed tigrolysis and pyknosis, and the Nissl granules were gathered into clumps. In the more advanced cases there was complete disintegration of the cell structure, and the position of the former cell was occupied by a structureless mass. Vacuoles were not observed. The authors noted that in the upper and lower portions of the nuclei of the oculomotor nerve, the appearance of the nerve cells was practically normal. They concluded that there was sufficient histologic change in the nerve cells to account for the disturbances of function which had been observed in the muscles of the eye.

Previous to 1915 all the descriptions of the lesions which are produced in botulism were based upon examinations of the tissues of animals in which the

poisoning had been experimentally produced. In that year, however, Paulus (19) reported a series of cases in which careful histologic examination was made of the brain and cord of one of the victims. The history of the illness was of some interest in that there had been transient symptoms of diplopia 3 weeks before the onset of the difficulty in swallowing, and death did not occur for 3 weeks after the latter symptom was first noted. Because of the fact that it is the first comprehensive report of the changes in the human brain, the report is given in some detail.

The brain was large and somewhat soft, and the meninges and brain were markedly congested. There was no evidence of meningitis or of hydrocephalus. On section, numerous hemorrhages up to the size of the head of a pin were seen in the substance of the brain, especially in the gray matter in the region of the ventricles, in the basilar nuclei, and in the floor of the fourth ventricle. In places in the stem the hemorrhages were confluent. The vessels of the ependyma were engorged. The spinal cord was hyperemic, and, in the cervical portion, contained a cystic cavity in the region of the central canal. There was no macroscopic evidence of destruction of any of the columns.

Microscopic examination showed marked congestion of all the vessels. In many places the hemorrhages occurred around the vessels, although the wall did not seem to be destroyed. The hemorrhages were most numerous in the region of the medulla and basal nuclei, and were most severe in the floor of the fourth ventricle. There was no evidence of inflammatory change, no round cell infiltration, and no destruction of tissue structure except where there was hemorrhage.

There were varying degrees of nerve cell changes in different portions of the brain, but the severity of the lesions did not seem to bear any relation to the number of hemorrhages which were adjacent. On the whole, they were most marked in the regions of the fourth ventricle and the aqueduct, where there were also numerous hemorrhages, but in places the most severe nerve cell changes occurred where there were no adjacent hemorrhages. Paulus' description of the changes in the ganglion cells is as follows:

"Die Ganglienzellen zeigen verschiedene Grade der akuten, teilweise auch der schweren Zellveränderung im Sinne Nissls: einfache trübe Schwellung, körnigen Zerfall der Tigroidschallen, Kernblähung, Auflösung der Kernmembran, Randständigkeit und Zerfall des Kerns, Auflösung des Zelleibs bis zum völligen Zellschwund. Vielfach liegen zwischen stark veränderten Zellgruppen vereinzelte, ganz normale Elemente."

The changes in the ganglion cells were especially marked in the region of the nucleus of the fourth nerve, and slightly less so in the nucleus of the third. In these areas a peculiar pigmentation was seen, in some places the pigment coexisting with the nucleus, and in other places completely replacing it. The nuclei of the vagoaccessorius group were also involved. The ganglion cells of the cord, especially in the cervical region, were markedly damaged, but the hemorrhages in the cord were more numerous in the posterior horns than in the anterior. In

neither the brain nor the cord were there any evidences of inflammatory processes, or of proliferation of the neuroglia cells. The columns of the cord were unimpaired.

Paulus described the lesion as a polioencephalitis superior and inferior, with acute degenerative hemorrhagic myelitis, or, in other words, as a polioencephalomyelitis hemorrhagica. He believed that the distribution of the lesions in the various nuclei was sufficient to explain the symptoms which were present during life.

Komotzki (139) investigated the changes which occur in the viscera in rabbits and guinea pigs in which botulism had been experimentally produced. He found marked congestion of all the organs, and an increase of leukocytes in the dilated vessels. In the liver there was marked dilatation of all the vessels, but especially of the veins and capillaries, and in many cases there was secondary atrophy of the adjacent liver cells. In about one-third of the cases there was fatty degeneration of the liver cells in the central portion of the liver lobules, and in a very few cases the fatty degeneration was diffuse and marked. It was never found exclusively at the periphery of the liver lobules. He noted that the toxin was extremely toxic for the liver cells; in the majority of cases there were miliary or larger necroses scattered irregularly throughout the liver tissue. In most of the necrotic areas there was complete destruction of the liver cells, including the nuclei, and in all there was infiltration with polymorphonuclear leukocytes. In some of the cases which survived for a longer time, from 11 to 49 days, there was evidence of regenerative processes around the necrotic areas, and in one the granulation tissue had invaded them. He did not find any evidence of specific toxin action on the cells of any other organs.

Bogomolez (140) investigated the changes in the adrenals of cats in which botulism had been produced. He found that there was an increase in secretory power of the medullary cells and believed that it was a compensating phenomenon to counteract the diminution of the cardiac muscle power. He also found an increase in the amount of lipoidal substance from the cortical cells of the adrenal, and he believed that this was of especial importance in view of the fact that Kempner and Schepilewsky (123) had observed that lipoids have an inhibiting action on the toxin of *B. botulinus*. Bogomolez advanced the hypothesis that the duration of life in *botulinus*-poisoning may depend upon the amount of lipoidal substance which is produced by the adrenals.

An important observation was made by Ophüls (99) in his study of the fatal case of the Stanford University series in California, the details of which have been previously recorded in this report. Ophüls found the usual hyperemia of the internal organs of the body, as well as bronchopneumonia and areas of collapse in the lungs. There were no macroscopic hemorrhages. Microscopic examination of sections from various portions of the brain showed marked congestion, and widespread thrombosis in the blood vessels, arteries, and veins of the meninges and of the brain. The thrombi were especially numerous in the stem and consisted of dense masses of fibrin in which many polymorphonuclear

leukocytes were enmeshed; there were very few conglutinated blood platelets. Perivascular hemorrhages were quite frequent in the meninges and in the brain tissue. There was no evidence of destruction of the ganglion cells of the various motor nuclei; the arrangement of the Nissl granules and the appearance of the nuclei were entirely normal. Examination of sections from the various internal organs showed one small thrombus in a vein of the submucosa of the small intestine and one in the ovarian plexus, but otherwise none was found.

The following description of the pathology of botulism is based upon a study of the tissues of 30 guinea pigs, 37 rabbits, 30 cats, 4 dogs, and 3 chickens, in which the poisoning was produced experimentally; of 6 chickens which died after eating the discarded string beans in Outbreak 4; and of 4 human cases in which I had the opportunity of making histologic examination.

The toxins which were used in the experimental work were derived from four strains of *Bacillus botulinus* which are described as Strains I, II, III, and IV. The animals were injected with toxins prepared by the growth of the bacilli in commercially prepared cans of string beans, and in infusions prepared from beef, pork, canned string beans, canned green peas, canned corn, canned asparagus, fresh artichokes, fresh peaches, fresh apricots, and crushed apricot stones. The virulence of the toxins varied greatly, that of Strains I and II being comparatively low, and that of Strains III and IV being very high. The symptoms of the animals and the type of the lesions which were produced differed only in degree, depending upon the amount of the toxin which was injected and the length of time which elapsed before the animals died.

The human cases from which the material was obtained consisted of one in which the brain was removed by the attending physician and was sent to Dr. Ophüls for examination; one in which the necropsy was performed by me; and two in which necropsy was performed by Dr. George B. Worthington, of San Diego, who sent me portions of the organs for examination. Two of the bodies had been embalmed before necropsy was performed.

The only constant finding on macroscopic examination of the bodies of victims of *botulinus*-poisoning is the marked congestion of the central nervous system and of the abdominal and thoracic viscera. In the central nervous system the meninges at the base of the brain, especially

around the pons and the medulla, are usually more markedly congested than at the cortex, and the basilar sinuses are usually engorged with blood. Not infrequently there are multiple hemorrhages around the base of the brain and the upper part of the cord. The tissues of the brain are also congested, and may be edematous and contain macroscopic hemorrhages (Fig. 7). The lungs are usually extremely hyperemic and may also show areas of collapse and of bronchopneumonia. The heart muscle may be opaque and flabby but shows nothing that is characteristic. There may be hemorrhages in the lungs (Fig. 8), and ecchymoses in the pleura, pericardium, and endocardium. The spleen is frequently enlarged, due to engorgement, and, when large, is apt to be soft. It may contain multiple hemorrhages. The liver and kidneys are usually cloudy and hyperemic; a considerable amount of blood escapes when the liver is sectioned. The vessels of the mesentery are engorged and the intestines are congested. Hemorrhages may be found in any of the abdominal organs but are especially frequent in the intestines. There is rarely any excess fluid in the pleural or peritoneal cavities.

At the site of subcutaneous injection in animals there may be local edema and hemorrhagic infiltration of the surrounding tissues, but there is never necrosis. The local reaction appears to depend rather upon whether filtered or unfiltered toxin has been injected, than upon the susceptibility of the animal, as it is more often found when the bacilli are injected with the toxin. The extreme, purulent reaction which van Ermengem (34, 86) described was never seen in my series.

The frequency with which hemorrhages occur varies considerably in the different kinds of animals. They may occur in the larger animals and in human beings, but are much more constant in guinea pigs and in chickens. In the smaller animals and in chickens there are practically always large hemorrhages around the base of the brain and upper part of the cord, whereas in the larger animals they are more rarely found, and, if present, are smaller. There were no macroscopic, meningeal hemorrhages in any of the human cases.

Microscopic examination of all the tissues shows that the congestion is even more marked than would appear from macroscopic examination. The veins are practically all engorged with blood, the capillaries are usually distended, and the arteries may remain filled. In

the spleen the greatest engorgement occurs in the medullary venous sinuses, and in the liver it is greatest in the intralobular veins and the adjoining capillaries. In the meninges and in the brain there are numerous minute hemorrhages around the distended veins, often without apparent break in the contour of the vessel wall; and in the brain, as in the other organs, there are often larger hemorrhages which are not confined to the immediate vicinity of the vessels. In some cases there is an excessive number of leukocytes in the blood in some of the vessels.

Thrombosis, of the type which was observed by Ophüls (Fig. 2), is extremely common; in fact the thrombi are so uniformly present and are so characteristic in appearance that they may be considered pathognomonic of botulism. Their appearance differs slightly in different animals, but in cats and dogs they are apparently identical with those found in human beings. The thrombi consist of dense masses of fibrin (Fig. 9) which is arranged in thick bands, and have many polymorphonuclear leukocytes enmeshed between the strands. They are frequently adherent to the sides of the blood vessels, but in a given section may be free from any connection with the vessel wall. In many cases there are no visible conglutinated blood platelets, but in some there are hyaline masses which are probably composed of platelets (Fig. 10). At times the red blood corpuscles have disappeared from the lumen of the vessel, as seen in cross-section, but in many cases they are intimately mixed with the leukocytes between the strands of fibrin.

The appearance of the earlier stages of the thrombus formation is not so characteristic. The blood vessels may contain a hyaline material, which may be conglutinated blood platelets, or there may be bunches of loose fibrin, frequently whorled, in which leukocytes and red blood corpuscles are enmeshed (Fig. 11). Not infrequently the hyaline material and the fibrin may coexist in one blood vessel, and the two may even be intimately mixed together. It is uncommon to find any leukocytes at this stage, but occasionally there may be large or small bunches of leukoctyes embedded in the hyaline material. It is probable that it was this stage of thrombus formation which was observed by Ossipoff (137).

There can be little doubt that this is an early stage of the thrombus

formation in the blood vessels; it is entirely different from anything that is seen in postmortem clotting, and may be found in tissues which are placed in fixing fluid within 1 or 2 minutes after the animal is killed. Moreover, it is not uncommon to find that in animals where numerous

TABLE I.

Frequency of the Occurrence of Thrombosis.

Material.	Animals lived.				Total.	
	1 day.	2 days.	3–7 days.	Over 1 wk.		
Guinea pigs.						
Thrombus	1	—	6	5	12	
Prethrombus stage	6	—	1	1	8	
No thrombus	4	—	3	3	10	30
Rabbits.						
Thrombus	2	1	7	11	21	
Prethrombus stage	7	2	—	—	9	
No thrombus	1	1	—	5	7	37
Cats.						
Thrombus	—	4	8	14	26	
Prethrombus stage	—	—	1	—	1	
No thrombus	—	1	—	2	3	30
Dogs.						
Thrombus	—	2	1	1	4	
Prethrombus stage	—	—	—	—		
No thrombus	—	—	—	—		4
Human beings.						
Thrombus	—	—	2	1	3	
No thrombus	—	—	1	—	1	4
Total	21	11	30	43	105	

Classification under "prethrombus stage" indicates that there was evidence of beginning thrombosis, as shown either by masses of loose fibrin within the blood vessels or by the presence of hyaline masses which are probably conglutinated blood platelets. There were no typical thrombi.

fully formed thrombi are present, there are some vessels which contain this incomplete stage of thrombus formation, and in some cases it has been observed that on one side of a vessel lumen there may be a fully formed thrombus, while on the other there is the incomplete form, the

two merging at the point of contact. For want of a better name I have called this early stage of the thrombus formation the "prethrombus stage" of the thrombosis.

The thrombi and the prethrombus structures are found in arteries and veins, though considerably more often in the latter. They may occur in the larger vessels which lie inside the organs, but are more frequent in the smaller branches which ramify within the tissues. They have never been observed in the blood which has escaped into the tissues, but it is common to find that there is a thrombosed vein in the vicinity of a hemorrhagic area.

There is some doubt as to the time which elapses before the thrombi are formed. Of eleven guinea pigs and ten rabbits which died within 24 hours after the administration of the toxin, one guinea pig and two rabbits showed fully developed thrombi in the vessels, and six guinea pigs and seven rabbits showed the earlier prethrombus formation. Of four rabbits, five cats, and two dogs which died on the 2nd day after inoculation, one rabbit, four cats, and both dogs showed the typical, fully formed thrombi, and none showed any of the prethrombus formation; and of sixty-nine guinea pigs, rabbits, cats, and dogs which survived for longer than 2 days, there were only thirteen which did not show fully formed thrombus or definite evidence of the earlier hyaline and loose fibrin formation. It would appear, therefore, that the formation of the thrombus begins during the first 24 or 48 hours of the intoxication, and that it reaches its full development very soon after that time. The relative frequency of the occurrence of thrombosis in the different animals is shown in Table I.

The difference between the thrombus which is usually found in the smaller animals, guinea pigs and rabbits, and that which occurs in the larger animals and in human beings, consists in the fact that the leukocytes are less numerous or even absent in the former. The difference is only relative, however, for in rabbits, especially, there may be accumulations of leukocytes in the thrombi, and in a few of the larger animals and in one of the human cases, the thrombi contained relatively few leukocytes. It is an interesting fact that, whereas the occurrence of thrombosis is relatively less frequent in guinea pigs and rabbits, that of large hemorrhages, especially in the meninges and brain, is relatively more frequent than in the larger animals.

The reason for the formation of the thrombus has not been determined. It was noted by the earlier authors, Kerner (7), Weiss (9), Schlossberger (13), and others, that the blood of victims of botulism remained fluid for a longer time than normally, but of this there is no recent proof. Van Ermengem (34, 86) recorded that the endothelial cells of capillaries were frequently degenerated, and, if this is true, it is reasonable to conclude that the thrombi, and also the hemorrhages, are secondary to the endothelial damage. However, in a short series of experiments in which the vital staining methods were used, I was unable to demonstrate any endothelial damage in the vessels in which thrombosis was present. The reason for the accumulation of leukocytes is also unexplained, and it is interesting that in none of the human cases in which blood counts were made, was there any marked actual or relative increase in the polymorphonuclear elements.

In a preliminary report (141) I recorded that thrombosis was frequently found in the blood vessels of the central nervous system. Subsequent investigation has shown that the thrombi are not confined to the central nervous system but that they are also found in other portions of the body. In general it may be said that thrombosis has been demonstrated in every organ where a systematic search has been made. Thrombi have been found in the meninges and tissues of the central nervous system, in the lungs (Fig. 4), heart muscle, liver (Fig. 12), kidneys, spleen (Fig. 6), adrenals, pancreas, and bone marrow. In a given case they may not be present in all the organs; they are most frequent in the vessels of the meninges, especially in the sulci, and in those of the lungs. In the kidneys, liver, and spleen they are comparatively common, and in the other organs they have been seen occasionally. They are perhaps more frequently found in the smaller veins, but are not uncommon in the large veins and arteries.

A careful examination was made of the nerve cells of the cranial motor nuclei and of the anterior horns of the spinal cord. In a few cases the tissues were placed in fixing fluid, absolute alcohol, within a few minutes after the animal was killed, and many sections were made from different portions of the brain and cord. The tissues of most of the other animals were placed in Orth's fluid within a few hours after death, and very few showed any appreciable amount of postmortem

degeneration. A few animals were treated with vital staining methods according to the procedures described by Kiyono (142) and Goldmann (143), in which carmine and trypan blue were used respectively. The tissues were fixed *in situ* by injecting 20 per cent formalin through the aorta, and were examined in frozen sections and paraffin preparations. Of the four human cases, necropsy was performed in two within 24 hours after death, and in two after the bodies had been embalmed. One of the latter cases had undergone so much post-mortem degeneration that practically all the nerve cells showed more or less disintegration.

In a few of the animals, especially in cats, there was some evidence of change in the nerve cells of the motor nuclei. The cells were shrunken, the contour was irregular, the protoplasm was opaque and sometimes vacuolated, and the nuclei stained poorly. Occasionally there were cells in which the normal structure was entirely lost, and there remained only an opaque, hyaline mass in the position which the cell had occupied. There was never any evidence of invasion by leukocytes or large phagocyte cells, or of proliferation of the neuroglia cells, as were described by van Ermengem (34, 86). In the majority of the animals, however, and in the three human cases in which the tissues were well preserved, there was no evidence of any change in the nerve cells which could be compared to those described by van Ermengem (34, 86), Marinesco (136), Ossipoff (137), Römer and Stein (138), and Paulus (19). The cells were of normal appearance, the nuclei were well stained and contained a nucleolus, and the granules of the protoplasm were arranged in the usual way.

There is some doubt as to how much of the damage in the nerve cells was due to artifact. The changes were very inconstant, were not localized to particular cell groups, and were not seen in those cases where the tissues were preserved immediately after death. Moreover, there was no evidence of pigment granules in the nerve cells of any of the animals which had been vitally stained, a point which is of considerable importance, since MacCurdy and Evans (144) have shown that when nerve cells are damaged before death, they may be easily recognized by the deposit of the pigment granules in their protoplasm.

The usual appearance of the brain and of the cord corresponds to that which was described by Ophüls in the fatal case of the Stanford

University series. There is marked hyperemia of the meninges and tissues, the veins being especially congested. Numerous small hemorrhages are seen in the region of the small veins in the meninges and the tissues, and not infrequently are situated in the immediate vicinity of a small vein, often surrounding it. The smaller hemorrhages are much more frequent in the brain than in the cord, and are scattered irregularly throughout the tissues of the cerebrum, stem, and cerebellum. The larger hemorrhages are more frequent in the stem.

Thrombi may be present in large numbers or may be difficult to find. They are most frequent in the vessels of the meninges, but are also present in those of the tissues. They are perhaps more common in the region of the stem, but are often found in the vessels of the pia mater in the sulci of the cerebrum and cerebellum, more frequently in the former than in the latter. An especially frequent situation for thrombosis, as well as for hemorrhage, is the space between the upper surface of the pons and the lower surface of the posterior portion of the cerebral hemispheres. The hemorrhages are often adjacent to veins which are filled with thrombus.

Reference has already been made to the frequency and distribution of the thrombi in the abdominal and thoracic viscera. The appearance of the lungs, heart muscle, adrenals, and gastrointestinal tract corresponds closely to that which was described by van Ermengem (34, 86, 87). The liver is congested, and shows varying amounts of parenchymatous and fatty degeneration of the liver cells, but the frequency of focal necrosis is much less, in my series, than was observed by Komotzki (139). The kidneys show marked parenchymatous degeneration of the cells of the convoluted tubules, which may be completely necrotic and desquamated. The cells of the straight tubules are less severely damaged, but the lumina contain casts of debris from the convoluted tubules. The congestion is chiefly confined to the veins and capillaries, but sometimes the glomerular tufts are engorged with blood. In a few of the animals and in one human case there were accumulations of exudate in the glomerular clefts (Fig. 13), and in some of them there were also red blood corpuscles. There was no demonstrable change in the epithelial cells of Bowman's capsule. In none of the organs were there any evidences of proliferation of the interstitial tissue or of infiltration with lymphocytes,

except in cases where it was evident that the proliferation of the interstitial connective tissue had preceded the intoxication. Some of the rabbits showed coccidial infections of the liver with proliferation of the interstitial connective tissue, and some showed some spontaneous chronic nephritis, but otherwise there was no evidence of interstitial tissue proliferation.

An interesting feature of a few cases was the occurrence of masses of cells in the lumina of the veins, especially in the liver. The cells were rhomboidal in shape, and had large, clear, vesicular nuclei. They were grouped in bunches of from 4 or 5 to 8 or 10, and were evidently desquamated endothelial cells.

RECORD OF EXPERIMENTS.

The experimental study of *Bacillus botulinus* was undertaken primarily to determine two points: (1) whether the organism is able to grow and to produce a virulent toxin in vegetable medium in which there is no protein of animal origin; and (2) whether the thrombosis which was observed by Ophüls (99) is a characteristic manifestation of poisoning with the *botulinus* toxin. A number of other problems have presented themselves during the course of the investigation, problems which deal with the method in which the toxin acts upon the tissues, but these have not been solved, and will not be discussed in detail.

Four strains of *Bacillus botulinus* were used in the investigation. Strain I was obtained from the American Museum of Natural History, New York; Strain II was supplied by Professor Hans Zinsser of Columbia University, New York; Strain III was isolated from the crop of a chicken which died after eating the beans in Outbreak 4; and Strain IV was recovered from the gizzard of a chicken which died after eating the spoiled corn in Outbreak 2. Another strain, No. V, was recently obtained from a can of string beans, one of the lot which caused the poisoning in Outbreak 7, but it has been studied only sufficiently to prove that it is *Bacillus botulinus*.

Experiments with Media Prepared from Vegetables and Fruits. Canned String Beans (Table II).

Twelve cans of commercially prepared string beans were opened by punching a nail-hole in one end. A small amount of fluid was removed for culture and for the injection of control animals, and about 1 cc. of a suspension of *B. botulinus* (Strain I) in normal salt solution was inserted. The nail-holes were closed with solder and the cans were kept at room temperature for from 2 to 12 months. The control cultures were all negative, but four of the control animals died within a few days, and although no bacterial cause of death could be established, the four suspected cans were discarded.

When the cans were reopened, aerobic slant agar, and deep glucose agar culture tubes were inoculated with the beans, the reaction of the fluid to phenol-

phthalein was determined, and guinea pigs, rabbits, and cats were injected with varying amounts of the fluid. In the majority of cases the fluid was injected subcutaneously, but in some it was injected into the peritoneal cavity.

TABLE II.

Results with Canned String Beans, Strain I.

Can No.	Date inoculated.	Date opened.	Odor.	Reaction.	Culture.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
	1914	*1914*									
8	Mar. 31	May 22	Good.	−	−	1			?	+	+
						2*		1*			
							1		3 wks.	+	−
							2		2 "	+	+
6	" 31	June 29	"	+3.2	+	3*	3*				
						4*	4*	2*			
								3	?	−	+
7	" 31	" 29	"	+2.5	−	5*	5*	4*			
						6*	6*	5*			
4	" 31	Nov. 10	"	+2.1	+	7*	7*	6*			
						8*	8*				
11	" 31	" 10	"	+1.7	+	9*	9*	7*			
						10*	10		2 days.	−	−
		1915									
1	" 31	Mar. 23	"	+2	+	11			6 wks.	+	+
							11		10 days.	+	+
							12		2 wks.	+	+
								8†			
								9	1 mo.	+	−
10	" 31	" 23	"	+2.2	+	12			6 wks.	+	+
						13*	13		1 wk.	+	+
							14*	10*			
								11	6 wks.	+	+
12	" 31	" 23	"	+2	+	14			2 mos.	+	−
						15*	15		10 days.	+	+
							16*	12	9 wks.	+	+
								13*			

* The animal showed no symptoms.

† The animal showed typical symptoms but recovered.

The reaction is stated in terms of the standard recommended by the American Public Health Association for the standardization of the reaction of culture medium. Phenolphthalein was used as the indicator.

The reaction of the contents of the cans was acid in every case, varying from 1.7 to 3.2 per cent; and pure cultures of *B. botulinus* were recovered from six of the cans.

The animals were kept under close observation for about 3 weeks and were then returned to the stock cages for used animals, as I was not aware that the appearance of symptoms of the poisoning might be delayed beyond that time, and that animals which became emaciated and died in from 6 to 8 weeks were, in fact, suffering from the effects of the toxin. It is therefore probable that several of the animals which are marked with an asterisk in Table II should be included among the positive cases, as they died and were discarded without my knowledge.

A number of the animals showed the typical symptoms of botulism, and post-mortem examination showed the macroscopic changes which were described by van Ermengem (34, 86, 87). Histologic examination showed multiple hemorrhages (Fig. 7) and thrombi (Figs. 9, 11, and 14) of the type which was observed by Ophüls (99) in almost all the cases. The distribution and appearance of the hemorrhages and thrombi have been discussed in another section of this report.

The investigation established three important facts concerning the toxin of *Bacillus botulinus:* (1) that it will develop in a medium consisting of cooked string beans to which no animal protein has been added; (2) that it will develop in a medium which gives an acid reaction to phenolphthalein; and (3) that when animals die from the effects of poisoning with the toxin, a peculiar type of thrombosis is to be found in the blood vessels of various portions of the body.

Infusions Prepared from Vegetables.

The infusions were prepared from canned string beans, canned peas, canned corn, canned asparagus, and fresh artichokes, respectively. In all, the method of preparation was the same. The material was boiled until it was soft and was then strained and kneaded through cheese-cloth. The reaction of the fluid was adjusted to about 0.2 per cent alkaline to phenolphthalein, and 1 per cent glucose was added. The medium was sterilized by fractional sterilization, and the cultures were covered with albolene and incubated in the dark at 28°C.

Infusion of Canned String Beans (Table III).—B. botulinus grew readily in the string bean infusion and produced a moderate amount of gas and the characteristic butyric acid odor. The experiments showed results similar to those

TABLE III.

Results with Infusion Prepared from Canned String Beans, Strains I and II.

Strain No.	Age of culture.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
I	11 days.	16*	17*				
I	?	17†	18‡		6 wks.	−	−
				14	4 "	+	+
I	16 days.		19*	15*			
				16	6 "	+	+
II	?	18			25 days.	+	+
II	18 days.		20		6 wks.	+	−

* The animal showed no symptoms.

† The animal showed symptoms but recovered.

‡ The animal was killed. There were no symptoms.

obtained in the series with canned string beans, and proved that Strain II is also able to produce a virulent toxin in medium prepared from string beans (Fig. 15). The addition of glucose to the medium and the adjustment to an alkaline reaction did not appear to cause any appreciable difference in the virulence of the toxin.

TABLE IV.

Results with Infusions Prepared from Canned Peas and Canned Corn, Strains I, II, and III.

Medium and strain.	Age of culture.	Amount injected.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
Peas.		*cc.*						
I	1 mo.	1.5	19			2 wks.	+	−
II	1 "	2	20			18 days.	−	+
II	1 "	1.5	21			6 "	+	+
II	1 "	5			17	5 wks.	+	+
II	1 "	4.5			18	17 days.	+	+
III	1 "	1			19	4 "	−	+
III	1 "	0.5			20	2 "	−	+
III	1 "	1	22			18 hrs.	+	P.T.
III	1 "	2		21		18 "	−	"
III	1 "	1			21	2 days.	−	−
Corn.								
III	1 "	1.5	23			18 hrs.	+	+
III	1 "	3		22		18 "	−	+
III	1 "	1			22	2 days.	+	+
III	6 wks.				23	3 "	+	+

P. T. indicates prethrombus stage.

Infusion of Canned Green Peas (Table IV).—The experiments of this series showed that *B. botulinus* will grow readily and will produce its toxin in a medium prepared from cooked green peas. In cultures of Strains I and II the virulence of the toxin was comparatively low, but in cultures of Strain III it was moderately high, the relative degree of virulence being approximately the same as occurs in cultures of the same strains in beef or pork infusion. In experiments with Strain IV, which are not yet completed, it was found that virulence of its toxin in peas medium was almost as high as in cultures of Strain III.

The odor of the peas culture is characteristic, there is much gas formation, the bacilli and spores are present in large numbers, and the toxin produces the typical symptoms and pathological changes in animals (Fig. 16). A chicken which was fed a small amount of the toxin developed the typical symptoms of botulism, which cannot be distinguished from those of limber-neck, and died within 36 hours.

Infusion of Canned Corn (Table IV).—The experiments showed that cooked green corn is also a suitable medium for the growth of *B. botulinus* and for the development of its toxin. The virulence of the toxin of Strain III was moderately high, and injections into animals, and feeding experiments in chickens showed that the typical symptoms and thrombosis are produced. The incomplete series

TABLE V.

Results with Strain III in Artichokes, Asparagus, Apricots, and Peaches.

Medium.	Age of culture.	Amount injected.	Guinea pig No.	Rabbit No.	Died.	Hemorrhage.	Thrombus
		cc.			*days*		
Artichokes.....	6 wks.	2	24		4	−	+
"	6 "	3		23*			
"	2 mos.	2	25		1	+	−
"	2 "	3		24	2	+	+
Asparagus.....	2 "	2	26		1	+	+
"	2 "	3		25	2	+	+
Apricots.....	6 wks.	2	27		7	−	−
"	6 "	3		26	19	+	+
"	2 mos.	2	28		3	+	P.T.
"	2 "	3		27*			
Apricot stones.....	2 "	2	29		1	+	P.T.
" "	2 "	3		28	2	+	+
Peaches.....	2 "	2	30		3	+	−
"	2 "	3		29	4	+	−
"	6 wks.	2	31		5	+	−
"	6 "	3		30*			

* The animal showed no symptoms.

P. T. indicates prethrombus stage.

of investigations with Strain IV showed that the toxin of that strain was also produced in a high degree of virulence. There was no investigation of Strains I and II in corn medium.

Infusion of Canned Asparagus (*Table V*).—The investigations with infusions of asparagus were confined to cultures of Strain III, and showed that a relatively virulent toxin is produced. The injected animals developed the typical symptoms of botulism and their tissues showed the characteristic thrombus formation and hemorrhages.

Infusion of Artichokes (*Table V*).—The investigation of artichoke medium was also confined to a study of cultures of Strain III, but they showed that the toxin may be formed in sufficient quantities to kill susceptible animals. The experiments were repeated at an interval of several months, and the results were the same in both series.

Infusions Prepared from Fruits.

The media from fruits were prepared in the same way as the infusions of vegetables, and fresh pears, peaches, and apricots were used. The stones of the apricots and peaches were crushed, and added to the fruit while it was boiling. One lot of medium was prepared from crushed apricot stones, from which all the fruit pulp had been removed. The preparation and incubation of the culture tubes were the same as in the vegetable infusion cultures.

Infusion of Pears.—Tubes of pear infusion were inoculated with Strain III of *B. botulinus* and allowed to incubate for over 2 months. Films from the tubes showed no evidence of the bacilli or spores, and injection into animals failed to produce any symptoms.

Four cans of commercially preserved pears were inoculated with Strain I and allowed to remain at room temperature for several months. When the cans were opened, pure cultures of *B. botulinus* were obtained from three of them, but injection into animals did not produce any symptoms.

Infusion of Peaches (*Table V*).—The investigation in peach infusion was confined to cultures of Strain III, and a single series of experiments was performed. The experiments showed that the toxin may be formed in peach infusion in sufficient quantities to produce the typical symptoms and thrombosis in susceptible animals. A series of cans of preserved peaches has been inoculated, but sufficient time has not elapsed for the completion of the investigation.

Infusion of Apricots (*Table V*).—Two series of experiments were performed with cultures of Strain III in apricot infusions, and in both it was shown that the toxin may be formed in sufficient quantities to produce the characteristic symptoms and lesions in animals.

The cultures in an infusion of crushed apricot stones developed a toxin which was even more virulent than that which was produced in an infusion of the whole fruit, and which caused the typical symptoms and pathological changes of botulism in guinea pigs and rabbits (Fig. 8).

The cultures of *B. botulinus* in cans of preserved apricots are not yet ready for study.

Experiments with Media Prepared from Pork and Beef.

The media that were used in this series of experiments were prepared according to the formulæ recommended by van Ermengem (34, 86) and Leuchs (114). The beef infusion (van Ermengem) contained 1 per cent sodium chloride, 1 per cent peptone, and 2 per cent glucose, whereas the pork infusion (Leuchs) contained 0.5 per cent sodium chloride, 1 per cent peptone, and 1 per cent glucose. The bacteria grew rapidly and developed virulent toxins in both, but no accurate comparative study was made of the toxin-producing power of the bacteria in the two media. The cultures were covered with albolene and incubated at 28°C. in the dark.

Experiments with Glucose Beef Infusion (Tables VI, VII, and VIII).—The growth of all the strains of *B. botulinus* in van Ermengem's medium was profuse,

TABLE VI.

Results in Glucose Beef Infusion (van Ermengem's Medium), Strains I and II.

Strain No.	Age of culture.	Amount injected.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
		cc.						
I	16 days.	2	32			7 days.	+	+
I	16 "	5		31‡		2 mos.	−	+
I	6 "	5	33			5 wks.	+	+
I	6 "	5	34			4 days.	+	+
I	7 wks.	3.5		32*				
I	7 "	5			24	6 wks.	−	+
I	5 "	3		33		24 days.	+	+
I	5 "	5			25	16 "	−	+
II	6 "	2	35†					
II	6 "	5		34		6 wks.	−	−
II	6 "	3	36			5 "	−	−
II	2 mos.	3.5		35		5 days.	+	+
II	2 "	5			26	2 mos.	−	−
II	2 "	3.5		36*				
II	2 "	5			27	2 mos.	+	+
II	2 "	5			28	15 days.	+	+

* The animal showed no symptoms.

† The animal showed symptoms but recovered.

‡ The animal was killed. There were no symptoms.

and the toxin production was relatively high. The medium became cloudy in from 48 to 72 hours, but within a week began to clear and the bacilli settled to the bottom of the tube. There was a moderate amount of gas formation and a very strong butyric acid odor. Toxin could be demonstrated in about 3 or 4

TABLE VII.

Results in Glucose Beef Infusion, Strains III and IV.

Strain No.	Age of culture.	Amount injected.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
		cc.						
III	25 days.	1	37			1 day.	−	−
III	25 "	3		37		1 "	−	P.T.
III	25 "	4			29	7 days.	−	+
III	16 "	F. 1	38			18 hrs.	−	−
III	16 "	F. 3		38		20 "	+	P.T.
III	16 "	F. 5		39		2 days.	+	"
III	16 "	F. 8 S.			30	4 "	−	+
III	16 "	F. 1			31	2 "	+	+
III	6 wks.	F. 0.001	39			18 hrs.	+	P.T.
III	6 "	F. 0.002	40			18 "	+	"
III	6 "	F. 0.0002	41			20 "	+	−
III	6 "	F. 0.0005		40		2 days.	+	−
III	6 "	F. 0.0055		41		4 "	−	+
III	6 "	1.5			32	5 "	+	+
III	6 "	1			33	5 "	+	+
IV	4 "	5			34	2 wks.	−	+
IV	6 "	2		42		18 hrs.	+	P.T.
IV	6 "	2		43		18 "	−	"
IV	6 "	2		44		18 "	−	−
IV	6 "	2		45		18 "	+	P.T.
IV	6 "	F. 2		46		18 "	+	"
IV	6 "	F. 1.5	42			18 "	+	"
IV	6 "	0.002		47		?	+	−
IV	6 "	0.004			35	3 days.	+	−
IV	6 "	0.001		48		20 hrs.	+	+

F. indicates filtered; S., repeated injections.

P. T. indicates prethrombus stage.

days, but it required at least 3 or 4 weeks for the maximum virulence to be reached.

The virulence of the toxins of Strains I and II in glucose beef infusions was extremely low as compared with that of Strains III and IV. A comparison of

Tables VI and VII shows that whereas in Strains I and II from 3 to 5 cc. of the infusion were necessary to produce death in animals in from several days to 6 weeks or 2 months, in Strain III 0.0002 cc. would kill a guinea pig, and in Strain IV 0.001 cc. would kill a rabbit within 24 hours. But in spite of the great differences in the degrees of virulence, the pathological lesions are identical in poisoning from all the strains, provided that the injections of the toxins of Strains III and IV are small enough to allow the animals to live for from 2 to 4 days or longer (Figs. 10, 12, and 17 to 20).

TABLE VIII.

Results of Injecting Dogs with Glucose Beef Infusion Cultures, Strains III and IV.

Strain No.	Dog No.	Age of culture.	Amount injected.	Died.	Hemorrhage.	Thrombus.
			cc.			
III	1	2 mos.	5	2 days.	−	+
III	2	2 "	F. 3	2 "	+	+
III	3	2 "	F. 1	3 "	+	+
IV	4	2 "	F. 2.3 S.	R.*		
III	5	2 "	F. 0.85 S.	16 days.	−	+

* R. indicates recovery. The animal was very ill for 2 weeks. Symptoms typical. The recovery was slow.

F. indicates filtered toxin; S., repeated injections.

Dogs have been found to be susceptible to the toxins of Strains III and IV in glucose beef infusion cultures. The susceptibility is comparatively low compared with guinea pigs and rabbits, and is slightly lower than that of cats, but the pathological changes are the same as in the other susceptible animals.

Experiments with Glucose Pork Infusion (Table IX).—Cultures of Strains I, II, and III were examined, and in all the toxin had developed. The toxins of Strains I and II were of very low virulence as compared with those of Strain III, and corresponded closely to those in the glucose beef infusion, but the toxin of Strain III was relatively low in comparison with the glucose beef infusion cultures of Strain III. The symptoms and pathological changes were the same as were produced in the other media.

TABLE IX.

Results in Glucose Pork Infusion (Leuchs Medium), Strains I, II, and III.

Strain No.	Age of culture.	Amount injected.	Guinea pig No.	Rabbit No.	Cat No.	Died.	Hemorrhage.	Thrombus.
		cc.						
I	8 days.	1.5	43†					
I	8 "	5		49*				
I	8 "	5			36*			
I	6 "	1	44			6 days.	+	+
I	6 wks.	3		50		17 "	+	+
I	6 "	5			37	2 mos.	+	+
I	6 "	3		51		20 days.	+	+
I	6 "	5			38	2 mos.	−	+
II	6 days.	2	45†					
II	6 "	4		52*				
II	6 "	5			39*			
II	6 "	1	46			9 days.	+	P.T.
II	6 "	3		53‡		5 wks.	−	+
III	6 wks.	5			40	2 days.	+	+
III	6 "	1 I.V.		54		5 "	+	+
III	6 "	3		55		8 "	+	+
III	6 "	1.5			41	7 "	−	+
III	7 "	1			42	5 "	−	+

* The animal showed no symptoms.

† The animal showed symptoms but recovered.

‡ The animal was killed. There were no symptoms.

P. T. indicates prethrombus stage.

I. V. indicates intravenous injection.

Experiments with Vital Staining Methods.

Four rabbits were injected with a solution of carmine, according to the method described by Kiyono (142), and were inoculated with a glucose beef infusion toxin of Strain III. All developed the typical symptoms of botulism, and their tissues showed the characteristic hemorrhages and thrombosis. There was no evidence of carmine granules in the endothelial cells of the blood vessels which contained thrombi, or in the nerve cells of the nuclei of the cranial motor nerves or of the anterior horns of the spinal cord.

Four cats and one rabbit were injected with trypan blue according to the method described by Goldmann (143), and were inoculated with a glucose beef infusion culture of Strain III. The animals were killed during an illness which was typically botulism, and the tissues were immediately hardened *in situ* by intraaortic perfusion with 20 per cent formalin. Microscopic examination was

made of frozen sections, and of paraffin preparations. The typical hemorrhages and thrombi were found in the tissues, but there was nothing abnormal in the endothelial cells of the vessels which contained thrombi, and there was no evidence of pigment granules in the ganglion cells of the central nervous system.

Experiments to Determine Whether There Is a Direct Action of the Toxin upon the Muscles.

In 1874 Pürckhauer (145) advanced the theory that the results of sausage-poisoning could best be explained by assuming that there is a direct toxic action upon the muscles, and that the disturbances in the musculature are not secondary to changes in the central nervous system. He drew attention to the fact that there is an inhibition of glandular secretions, and believed that these could also be explained by the direct action of the toxin upon the secreting cells. He argued that the order in which the symptoms of botulism develop, and the differences that are found in the mild and severe cases afford strong support to his theory that the muscles are directly affected. In the milder cases there may be only disturbances of vision due to paralysis of the intrinsic muscles of the eyes, and in the more severe cases the eye symptoms usually appear first. Involvement of the extrinsic muscles of the eyes, of the muscles of the pharynx, larynx, and gastrointestinal tract appear later. In the more severe cases there is weakness of the heart muscle and of the skeletal muscles, and in fatal cases death is frequently due to cardiac failure.

Pürckhauer attempted to explain the regularity with which the symptoms develop in a definite sequence, by assuming that the damaging effect of the toxin is first manifested in the more delicate muscles of the body, and that the order in which the larger muscles are damaged is dependent upon their size. The extremely delicate muscles of the iris are therefore among the first to suffer, and any interference with their action is immediately recognized by the patient. The muscles of the orbit, pharynx, larynx, and gastrointestinal tract are relatively small and are also damaged early, but the heart muscle, which is large, is affected comparatively late. He believed that the skeletal muscles would also lose their function if the toxin could act sufficiently long, but he pointed out that the patients usually died from cardiac failure before the skeletal muscles became involved. He drew attention to the fact that there is an absence of facial expression even when there is no paralysis of the facial nerve, and he believed that this, as well as the general muscular weakness, was an indication of a definite lesion in the muscle tissue. He recalled that Kerner had described excessive rigor mortis in the bodies of patients who had died of sausage poisoning and he believed that this was also evidence that the muscles had been damaged.

A short series of experiments was performed to determine whether the toxin of *Bacillus botulinus* has any direct action upon muscle and

other types of protoplasm. An extremely powerful toxin of Strain III was used, and the experiments were all thoroughly controlled.

Experiment 1.—Different varieties of bacteria were grown in association with *B. botulinus* in glucose beef infusion culture, and were allowed to remain until a highly virulent toxin was developed. There was no apparent damage to the associated bacteria.

Experiment 2.—Strips of frogs' pharynges were placed in a filtered infusion in which there was a virulent toxin, and the activity of the ciliary movement was determined by placing small pieces of cork upon the surface and estimating the distance they were carried within a certain time. There was no inhibition of ciliary activity in the strips which were immersed in the toxin, and their movements were continued for as long a time as in control strips which were placed in a similar infusion which did not contain any toxin.

Experiment 3.—The gastrocnemii muscles of a frog were placed in parallel in an electric circuit, with the terminal wires embedded in the muscle. One muscle was immersed in a filtered glucose beef infusion culture of *B. botulinus* and the other was placed in glucose beef infusion (of the same lot as the culture) in which there was no toxin. The muscles were fatigued by intermittent stimulation with a tetanizing current which was of equal strength in both muscles. The muscle which was immersed in toxin did not fatigue any sooner than the control, and after a period of rest it responded fully as well as did the control.

Experiment 4.—Small muscles from the thighs of a guinea pig were arranged in the same manner as were the frogs' muscles, and were kept at body temperature by immersing the apparatus in a water bath. The results of stimulation were the same as in the experiments with frogs' muscles.

Experiment 5.—Two guinea pigs of equal weight were selected, and one was injected with a lethal dose of the *botulinus* toxin. When the animal was so ill that it was unable to use its hind legs, corresponding muscles were taken from the thighs of the poisoned and the normal guinea pigs and suspended in modified Locke's solution in the same manner as in the previous experiments. The muscles were kept at body temperature by immersing the apparatus in a water bath. The muscle of the poisoned animal did not fatigue any sooner than the muscle of the normal animal, and after a period of rest it responded fully as well as did the other.

The investigation was not pursued further, as it seemed clear that the toxin of *Bacillus botulinus* (Strain III) had no direct poisonous action upon the protoplasm of bacteria, frog epithelial cells, frog muscle, and guinea pig muscle.

CRITICAL REVIEW.

The important facts that have been established by my investigations are four in number: (1) that botulism is endemic in the United States and is of comparatively frequent occurrence on the Pacific Coast; (2) that the toxin of *Bacillus botulinus* may form in a medium which is of purely vegetable composition; (3) that there is apparently a close relation between the botulism of human beings and a certain, hitherto unexplained, illness of domestic fowl; and (4) that the *botulinus* toxin produces characteristic lesions in the body, in the form of thrombosis in the blood vessels of many of the organs.

Previous to 1913, there was only one report in the available American literature for 20 years in which the diagnosis had been made, but a review of the literature on food-poisoning revealed three other outbreaks in which the illness was probably due to the *botulinus* toxin. Since the beginning of 1913 there have been twelve recorded outbreaks of food-poisoning in which the symptomatology of botulism has been recognized, and of these twelve series of cases, eight have occurred in California and one in Oregon. There is little doubt that many other outbreaks of botulism have passed unrecognized during this time. I have incomplete records of a case in Hornbrook, California, in which a woman died of "bulbar paralysis" and nearly 100 of her chickens died from limber-neck within a few days. Dr. E. F. Holbrook of San Jose, California, told me that he had seen two similar cases before he treated the one which I have recorded (Outbreak 4), and Dr. Bine and Dr. Lartigau of San Francisco have treated three patients who were probably cases of botulism. Dr. Phillips of Palo Alto recalls five cases of poisoning from canned beans which he saw in Amador County, California, and Curfman referred to five similar case which were observed in Colorado. In these, and probably in many other similar cases, a diagnosis of botulism was not made, and the Bureaus of Vital Statistics contain few records of cases in which botulism was given as the cause of death. If, however, it were possible to trace all the cases in which "ptomaine poisoning" has been

given as the cause of death, I have little doubt that botulism would be found to occupy an important position in the mortality list of the preventable diseases.

The result of the investigation which has the greatest economic importance is the demonstration that botulism may be produced by the ingestion of spoiled canned vegetables and fruits. This type of food-poisoning has been considered to be one of the specific meat intoxications, and of the five reported outbreaks which occurred in this country previous to 1914, four were attributed to poisoning by food of animal origin. Of the eleven more recent outbreaks of botulism, however, there is only one in which the poisoning was traced to meat. In three the source of the poison was not determined, but in seven it was definitely traced to home-canned vegetables or fruit, and in one it was traced to commercially canned vegetables. Four of the outbreaks were due to poisoning by canned string beans, one by canned green corn, one by canned asparagus, and one by apricots which had been canned without sugar.

In the cases which were recorded by Wilbur and Ophüls and in those of Outbreak 1 of my series, there seemed to be no doubt as to the diagnosis of botulism, as the clinical picture was complete; but it was not until the occurrence of Outbreaks 2 and 4, in December, 1915, and January, 1916, respectively, that the diagnosis was fully established by the recovery of *Bacillus botulinus* from remnants of the discarded food. More recently, in February, 1917, a third strain of *Bacillis botulinus* was recovered from a can of string beans which was of the same lot that had been responsible for the poisoning in Outbreak 7; and one can therefore conclude that the poisoning from the vegetables is, indeed, dependent upon the toxin of *Bacillus botulinus*.

In all the recorded cases in which canned vegetables or fruits have been responsible for the poisoning, the food has not been cooked after it was removed from the can. Three of the victims in my series tasted small portions from the cans of string beans and corn to determine whether they were spoiled, and one drank the fluid from a jar of asparagus. In two instances the canned string beans were served as salad, and in one the apricots were served as dessert.

The fact that boiling will destroy the toxin was illustrated in Outbreak 3, where the mother died after drinking the fluid from the freshly

opened can of asparagus, and the son escaped illness although he ate a portion of the asparagus after it had been cooked.

The virulence of the toxin in vegetables is very great in some cases. Two women died after merely tasting string beans and corn, respectively, to determine whether they were good, and one woman became ill after tasting beans. In Outbreak 1 all the patients died after eating home-canned apricots, and in Outbreak 7 four died and three were ill after eating string bean salad. In Wilbur and Ophüls' series, eleven out of twelve patients recovered after eating string bean salad, but with the exception of this one series, all the outbreaks which have been caused by vegetables or fruits have shown an extremely high mortality.

My experimental work has shown that the strains of *Bacillus botulinus* which were isolated from the vegetables are not different in their action from stock strains which were obtained in New York, and which, presumably, were recovered from meat. The stock strains, I and II, produced virulent toxins in medium prepared from string beans and green peas, and the symptoms and pathological changes that were produced in animals were identical with those produced by my newly isolated strains, III, IV, and V. The experiments have also shown that various fruits and vegetables may be suitable media for the development of the *botulinus* toxin, as positive results were obtained in media prepared from string beans, green peas, green corn, artichokes, asparagus, apricots, and peaches.

It is a point of considerable importance that foodstuffs which are contaminated with the toxin of *Bacillus botulinus* may not appear sufficiently spoiled to ensure their being discarded. The vegetables usually have an unpleasant odor and may show bubbles of gas on the surface, but they are not apt to be discolored or soft, and may even appear to be especially well preserved. It should be thoroughly understood that an extremely virulent toxin may produce but little change in the appearance of the food, and the common practice of tasting canned stuff to see whether it is fit for use should be discouraged. All canned food should be discarded if there is any indication that it is even slightly spoiled (this is even more important with home-canned food), and under no circumstances should it be eaten or even tasted before it has been cooked.

That the method which is usually employed in canning vegetables and fruits at home is not efficient is proved by the frequency with which a larger or smaller proportion of the finished product becomes spoiled. The average housewife knows nothing concerning the habits of bacteria or of the significance of the spores in spore-bearing bacteria; and she has no conception of the importance of thorough sterilization. With the older method of preserving with sugar the danger was not so great, as it is probable that even a moderate amount of sugar inhibits the development of the toxin, but in the process of canning without sugar the greatest care is necessary to ensure that all the bacteria and spores are destroyed.

The usual method which is adopted in the home-canning process is somewhat as follows: The jars or cans are prepared by filling them with boiling water and allowing them to stand for a few minutes; it is seldom that they are kept in boiling water for a time sufficient to kill any spores that may be present. The fruit or vegetables are washed, and are placed in the jars, often without having been previously cooked, as it is known that too much boiling will cause the material to soften and to lose its fresh appearance. The jars are filled with water and placed in a large container, frequently a wash-boiler, and allowed to steam for 2 or 3 hours, or perhaps longer. They are then removed from the boiler, sealed, and stored away.

Such a process of canning is entirely inadequate when no sugar or other preservative is added to inhibit bacterial action. The amount of moist heat that is necessary to kill bacterial spores varies with the different varieties of bacteria and many spores will withstand a temperature below that of the actual boiling point for a considerable length of time. Wolffhügel and Hueppe (146) investigated the cause of the spoiling of canned meats which had been presumably sterilized after being canned, and they found that if a can which contained 2,735 gm. of meat was kept immersed in boiling water for 3 hours, the temperature in the center of the can did not rise above 92°C.; and that if a can one-quarter as large was treated in the same way the temperature in the center did not rise above 98°C. Many bacterial spores will withstand this temperature for a considerable length of time, and in the method of sterilization which is in common use by the housewife, it is probable that the temperature in the center of the jars is not even as high as was obtained by Wolffhügel and Hueppe.

The United States Department of Agriculture[6] advocates that the method of fractional sterilization should be used in the canning of fruits and vegetables at home, but the advice is not generally heeded. However, fractional sterilization, as it is usually carried out, is not sufficient to kill the spores of *Bacillus botulinus*, as was shown in the report of Wilbur and Ophüls (99). The instructions for fractional sterilization of canned goods direct that the jars should be left unsealed until after the last sterilization, in order that the spores may germinate and thus become susceptible to the second and third applications of heat. *Bacillus botulinus* is a strict anaerobe, and if the jars are left open in the interim between sterilizations the spores will not generate, and are therefore not susceptible to the later process of sterilization. It is only with steam under pressure that the spores of *Bacillus botulinus* can be surely destroyed, and it is doubtless due to their efficient methods of sterilization that the commercial canners of foods have not been troubled with this form of food-poisoning in vegetables. A number of the housekeeping magazines are advocating the use of small autoclaves in the home kitchen, and if these are used the danger of infection with *Bacillus botulinus* will be greatly reduced; but with the methods of home-canning which are now in general use, the process is always attended with danger of food-poisoning of this virulent type.

The demonstration that domestic fowl, chickens and turkeys, develop an illness which in every way corresponds to limber-neck is also of interest. Pürckhauer (145) recorded that ducks had been poisoned after eating remnants of the poisonous sausages which had caused the illness of his patients, but van Ermengem (34, 86) reported that chickens are not susceptible, although pigeons are extremely susceptible to the toxin of *Bacillus botulinus*. In four of the outbreaks which are reported in my series, in the one which was recorded by Sheppard, and in the suspected outbreak at Hornbrook, California, varying numbers of chickens died after an illness in which the symptoms were those of limber-neck. Moreover, in the two instances in Escondido, California, where spoiled string beans were thrown out,

[6] Experiment Station Work XXVI, prepared by True, A. C., *U. S. Dept. Agric., Farmer's Bull. 262*, 1906. Breazeale, J. F., Canning vegetables in the home, *U. S. Dept. Agric., Farmer's Bull. 359*, 1909.

and in one at Hanford, California, where spoiled corn was discarded, numbers of chickens, and, in one instance, several turkeys, developed the same symptoms after eating the discarded food. Limber-neck is supposed to be due to the ingestion of spoiled meat which is fed to the chickens, but it is known that chickens and turkeys can eat putrefying meat without suffering any injury. My experiments have shown that the typical symptoms of limber-neck may be produced by feeding chickens with the toxin of *Bacillus botulinus*, and it is reasonable to suppose that even when the symptoms follow the ingestion of spoiled meat, it is *Bacillus botulinus* which is responsible for the poisoning.

In this connection it is interesting to note that Saunders (147) has attempted to correlate poliomyelitis in human beings and limber-neck in chickens, and that he believes that the two are identical. In an elaborate series of experiments he found that the larvæ of the green fly (*Lucilia cæsar*) are capable of transmitting a virulent toxin, which he believes to be the toxin of poliomyelitis, within 3 days after the fly had become contaminated from carrion flesh, and he concluded that poliomyelitis in human beings is due to ingestion of larvæ from an infected fly. Saunders recorded series of cases in which horses, mules, cattle, sheep, hogs, opossums, and buzzards had been known to develop paralysis at the same time that chickens were affected with limber-neck, and he attributed the cause for the development of the illness to the ingestion of infected carrion and to the activity of the green fly. He mentioned that the poisoning could not be due to the *botulinus* toxin because "the dog is absolutely resistent to the *botulismus* toxin," and recorded specific instances in which dogs had contracted the disease after eating the carcasses of chickens which had died of limber-neck.

My experiments have shown that dogs are not immune to the action of the *botulinus* toxin, and there are at least two recorded cases in which dogs became paralyzed after eating the food which had caused botulism in human beings.

In 1866 Niedner (106) recorded that a dog became paralyzed and died after eating the remnants of a poisonous sausage, and in the record of Outbreak 1 of this report it is stated that a dog became paralyzed after eating a portion of the discarded apricots, material which was responsible for the production of botulism in five persons, and of limber-neck in a number of chickens. In a recent report Buckley and Shippen (148) recorded that dogs are not susceptible to large doses of the toxin of the strain which was isolated from cheese in the New England States, but they administered the toxin by mouth only, whereas I used subcutaneous injections exclusively. Further study is therefore necessary before it is certain whether there is actually a difference in the effect of the two toxins.

There is no record that hogs are susceptible to the toxin of *Bacillus botulinus*; in fact in Outbreak 2 it was noted that a number of hogs which ate a portion of the discarded corn failed to show any symptoms of illness, although a large number of chickens which also ate some of the corn developed limber-neck, and died.

Curfman (103) recorded that a number of burros which ate the remnants of the food which had caused the poisoning in his series, died after showing similar symptoms to those that were observed in the human cases, and Buckley and Shippen (148) reported that they had succeeded in producing an illness somewhat analogous to forage-poisoning in horses and donkeys. Leuchs (114) and Wassermann (149) have shown that horses are susceptible to botulism and that they may be used in the production of antitoxic sera, and Kempner (128) showed that goats are also susceptible. Schneidemühl (150) believed that the milk sickness of calves is due to the action of the *botulinus* toxin, but in so far as I have been able to learn, his theory has not been confirmed.

Whether the paralysis which Saunders has described was due to the toxin of *Bacillus botulinus* must remain an unsettled question. It is certain, however, that the *botulinus* toxin produces symptoms of paralysis in human beings, monkeys, horses, goats, dogs, cats, rabbits, guinea pigs, white mice, turkeys, chickens, and pigeons; and it is an interesting fact that Bail (151) has shown that various species of flies are capable of acting as carriers of *Bacillus botulinus*.

The occurrence of thrombi in Wilbur and Ophüls' fatal case (99) and in three of the fatal cases of my series, and the uniformity with which thrombi are found in animals which have died from experimentally produced botulism, establish beyond all possibility of doubt that thrombosis is a pathological process which is characteristic of poisoning with the toxin of *Bacillus botulinus*. But as to the cause of the thrombus formation and the importance of the thrombi, in so far as the clinical manifestations are concerned, very little is known at the present time.

A striking feature of the clinical course of botulism is the uniformity with which the signs of involvement of the central nervous system always develop in the same sequence, whether the poisoning is so severe that it rapidly progresses to a fatal termination, or whether it is so mild that only the initial signs and symptoms are produced. The muscles that receive their motor nerve supply from the cranial motor

nerves are the ones which suffer most, and it is not uncommon, indeed it is the rule, to find partial or complete paralysis of some or all of them. On the other hand, it is extremely uncommon to find paralysis of muscles which receive their motor nerve supply from the spinal cord, although there is usually incoordination of muscular movement and very marked muscular weakness. The muscles which are supplied by the third cranial nerve are the first to be affected and the initial neurogenic symptoms are usually disturbances of vision. The fourth cranial nerve may escape injury, but usually the fourth and sixth are very early involved. Impairment of the ninth, tenth, and twelfth nerves is soon evident, and the patients complain of difficulty in moving the tongue, and in talking and swallowing. The onset of constipation, which is probably partly dependent upon involvement of the vagus nerve, may be delayed for several hours after the appearance of the eye symptoms. Impairment of function of the muscles supplied by the fifth and seventh nerves is less frequently observed, but is not rare. In very mild cases the neurogenic signs may not develop beyond impairment of the muscles supplied by the oculomotor nerve, but in the more severe the time which elapses between the various manifestations may be very short. The clinical picture is essentially that of a bulbar paralysis, with the earliest symptoms indicating injury high up in the brain stem, and death usually is caused by respiratory or cardiac failure.

The sensory nerves and the mentality of the patients are usually unimpaired. There may be some disturbance of the sense of taste but this is probably largely due to the inhibition of the salivary secretion, and disturbances of the olfactory, optic, and auditory nerves are very rare. The patients frequently complain of dizziness and headache, but otherwise the sensorium is not affected.

Metabolism is apparently inhibited as the temperature is usually subnormal and the patients are extremely susceptible to cold. The rapid emaciation may be partly due to the effect of the toxin, but is largely dependent upon the fact that the patients are unable to swallow food.

A satisfactory explanation for this peculiar combination of symptoms and signs has not yet been established. Kerner (7) believed that the toxin acted primarily upon the sympathetic nervous system, to which it was carried directly

from the stomach. He stated that there was no effect upon the muscles which were supplied by nerves which come directly from the brain, even though they pass through the thorax, where the sympathetic system was most severely damaged, but that the signs of involvement were constantly found in muscles in which the nerve supply is closely associated with branches of the sympathetic system. Weiss (9) believed that the action of the toxin was not primarily exerted upon the sympathetic system, but that the changes in the central nervous system were secondary to changes that were produced in the blood, and Müller (81) stated that the majority of authors agreed with Weiss. Pürckhauer (145) thought that the manifestations of botulism could be more readily explained by assuming that the toxin acted directly upon the protoplasm of the muscle cells and glandular secreting cells, and that the central nervous system was not primarily involved.

Marinesco's (136) demonstration of degeneration processes in the nerve cells of the motor nuclei in animals which had succumbed to botulism, appeared to have furnished anatomical proof that the paralysis is dependent upon lesions which are produced in the motor ganglion cells. His observations were confirmed by several authors. Ossipoff (137), Kempner and Pollack (115), and Römer and Stein (138) found similar changes in the brains and cords of animals in which they produced the intoxication experimentally, and Paulus (19) observed them in the brain of a person who had died from botulism. These authors believed that the toxin of *B. botulinus* has some specific affinity for the ganglion cells of cranial motor nuclei, and that the paralysis of the specific groups of muscles is thereby explained.

There are certain points, however, in which the deductions which were drawn by Marinesco and those who followed him, are not conclusive. Marinesco (136) observed that the greatest damage occurred in the cells of the anterior horns of the spinal cord, and that the cells of the medulla and pons were less severely damaged. He found some change in the nerve cells in the peduncles, but none was seen in those of the cerebral hemispheres. Ossipoff (137) agreed that the nerve cells of the spinal cord were more seriously damaged, especially in the regions which supply the muscles of the extremities, but he found that the Purkinje cells of the cerebral hemispheres were also changed.

The distribution of these lesions, as described by Marinesco and Ossipoff, does not correspond to the distribution of the motor paralysis or to the order in which the various muscle groups are affected. The earliest clinical sign, and one of the most constant, is due to the involvement of the third cranial nerve, the nucleus of which is in the region where Marinesco found the least severe nerve cell changes. It is true that Römer and Stein (138) found changes in the cells of the unpaired median nucleus of the third nerve, and it is unfortunate that they did not record the relative amount of cell change in that area and

in the spinal cord, but Ossipoff and Marinesco agreed that the lesions in that part of the brain stem are less severe than in the cord. Moreover, the muscles of the trunk, especially of the extremities, are rarely paralyzed, although the most severe nerve cell lesions occur in the portions of the cord which supply them, and it is difficult to understand why, if the nerve cells which are most severely damaged, do not produce paralysis of their corresponding muscles, it should be assumed that those in which the lesions are less severe should be held responsible for the paralysis of the muscles which they supply. Kempner and Pollack (115) observed the nerve cell changes in the animals which they examined but could not find any relationship between the severity of the clinical symptoms and the degree of disintegration in the nerve cells. Moreover, they noted that the lesions of the cells were still demonstrable for several weeks after the clinical symptoms had entirely disappeared, and it is difficult to understand how this could be if the muscular paralysis is dependent upon the changes in the nerve cells. In anterior poliomyelitis there is an analogous condition to that which was assumed to be the case in botulism, in that the muscular paralysis is dependent upon destruction of the motor nerve cells of the anterior horns of the spinal cord, but in poliomyelitis the effects of the nerve cell damage persist after the patient recovers from the acute illness, and certain muscles or groups of muscles remain paralyzed. In patients who have recovered from severe intoxications with the *botulinus* toxin, there is no persisting paralysis of the affected muscles—another fact which raises doubt as to the accuracy of the deductions of Marinesco and his followers.

Ophüls' (99) observation that the blood vessels of the brain and meninges contained thrombi which were more numerous around the base of the brain, appeared to give sufficient anatomical explanation for the symptoms that were observed, and were of especial interest because the nerve cells of the basal ganglia did not show any of the changes which were described by Marinesco and others. The thrombi seemed to give sufficient anatomical explanation for the distribution of the muscular involvement and for the peculiar variations in the severity of the symptoms which were observed in his case. A study of the fatal human cases of my series would seem to bear this out, as in all but one there were numerous thrombi in the blood ves-

sels and an absence of changes in the structure of the nerve cells of the cerebral motor nuclei.

At the time that I published my preliminary report (141), my observations had been confined to the effects of a toxin of relatively low virulence, which did not cause the death of the animals until there had been sufficient time for the full development of the thrombi. The almost constant thrombosis in the blood vessels of the brain led me to believe that the whole clinical and pathological picture, in so far as the central nervous system was concerned, was to be explained by the presence of the thrombi which obstructed the blood vessels, and that the disturbances of the function of the muscles and the occasional lesions in the nerve cells were dependent upon an insufficient flow of blood to the brain tissue. But even then it was difficult to understand why the symptoms shou d always appear in practically the same order and why there is no involvement of the sensory nerves. It was easy to believe that in the more severe intoxications, the severity of the symptoms and the fatal termination could be explained by a more extensive and rapid formation of thrombi in the region of the vital centers, but it was difficult to explain why the region of the nuclei of the third cranial nerve should always be the first affected, and why there is never any break in the sensory fiber tracts. The distribution of the thrombi is not confined to any restricted portion of the body, but occurs in all the organs, and in the meninges of the cerebrum, cerebellum, and cord as well as around the brain stem. It is therefore inconceivable that the vessels in the region of the oculomotor nuclei, on both sides, should always be the seat of the earliest thrombus formation, and that only the motor nuclei should be involved in the secondary ischemic degeneration.

When I obtained Strains III and IV of *Bacillus botulinus*, each of which produces a highly virulent toxin, which, in minute doses, will kill a guinea pig or a rabbit within 24 hours, it soon became evident that the animals could develop extremely severe symptoms which were quite typical, and die, and not show any thrombosis in the blood vessels of the brain or meninges. In many, it is true, there was evidence of what has been described as the prethrombus stage of thrombosis, but this was evidently not sufficient to cause an obstruction of the flow of blood that would lead to degeneration of the

tissues of the brain. In other cases, moreover, no evidence of the prethrombus stage could be found, and the only conclusion that could be drawn is that the symptoms of the *botulinus* intoxication are dependent upon some other factor than is indicated by anatomical change in the motor ganglion cells, or by thrombosis in the blood vessels which supply them.

Just what this other factor is remains unexplained. It is, of course, possible to say that there is some specific selective action which affects certain groups of the motor ganglion cells, but that is, to say the least, an unsatisfactory explanation. It is possible that the toxin acts, as does belladonna, upon the terminal end-plates of certain nerves, and the close resemblance between the effects of the *botulinus* toxin and those of the administration of belladonna suggest that this may be true. In cases which have been poisoned with the toxin of *Bacillus botulinus*, the pupils are dilated, the secretions are diminished, and the movements of the gastrointestinal tract are inhibited, and, moreover, the administration of eserine will cause a contraction of the dilated pupil, and pilocarpin will cause the salivary secretion to be reestablished, although the effect of both drugs is only temporary. But in *botulinus*-poisoning there is paralysis of striated muscle fibers as well as of unstriated fibers, although only certain groups of striated muscle fibers are involved, and it remains to be shown whether it is possible that the toxin may produce such a peculiar combination of effects by acting on the terminal nerve endings or on the synapses in the course of the peripheral nerve fibers.

The action of the toxin on the blood vessels is marked and constant. The vessels are widely dilated, and, in addition to the thrombosis, there are numerous hemorrhages in various portions of the body. Ophüls (99) suggested that the dilatation of the blood vessels might be due to the action of the toxin on the smooth muscle fibers in the walls, either directly, or through the medium of the vasomotor nerves. It seems probable that the latter explanation is correct. The presence of hemorrhages indicates that there is some direct damage to the endothelium of the blood vessels, and this, together with the slowing of the blood stream, due to the dilatation of the vessels, may explain the tendency to thrombosis. It does not explain, however, the peculiar cellular type of the thrombus which usually is packed with polymorphonuclear leukocytes.

No characteristic lesions, other than the hemorrhages and thrombosis, were found in the abdominal and thoracic viscera. The appearance of the tissues was as van Ermengem described, except that there was no evidence of an inflammatory process. The liver and kidneys showed parenchymatous degeneration, and in some instances there was evidence of hemorrhage into the glomerular capsule, but there was no evidence of any process which was secondary to the thrombosis. In the lungs the thrombi were especially numerous, and they were usually associated with areas of bronchopneumonia, but they may occur in lungs in which there is no pneumonia and there is no apparent causal relationship between the two processes.

As a result of my investigation, therefore, I have been forced to conclude that the method in which the toxin of *Bacillus botulinus* acts upon the tissues is unknown. It is probable that there is a direct damage to the endothelial cells of the blood vessels, and that the hemorrhages and thrombosis are secondary to that damage. It is also probable that when degeneration occurs in the cells of the central nervous system, it is an ischemic degeneration due to the presence of thrombi in the blood vessels which supply the part. But the evidence is against the explanation that the clinical symptoms are dependent upon the lesions which Marinesco described in the nerve cells of the brain and cord, or that they are secondary to thrombosis in the vessels of the central nervous system.

CONCLUSIONS.

1. Botulism is endemic in the United States and is comparatively common in the Pacific Coast States.

2. It is not essentially a meat poison but may also occur in canned vegetables and fruits.

3. The methods which are usually employed in the home-canning of vegetables and fruits are unsafe.

4. All home-canned vegetables should be cooked before they are eaten.

5. Botulism is a frequent cause of the so called limber-neck of domestic fowl, and it may be responsible for certain types of paralysis of various kinds of domestic animals, including dogs.

6. The occurrence of limber-neck in domestic fowl, if it has developed after they have eaten refuse from the kitchen, may be an indication for the prophylactic administration of the *botulinus* antitoxin to all persons who have eaten the suspected food.

7. Thrombosis in the blood vessels of various organs of the body is a characteristic lesion which is produced by the action of the toxin of *Bacillus botulinus*.

8. The cause of the symptoms of botulism and the way in which the paralysis of the various muscles is produced are unknown.

9. The normal habitat of *Bacillus botulinus* and the way in which the vegetables become contaminated are unknown. It is possible that the bacillus is normally present in the intestinal contents of the hog, and that the vegetables become infected when hog manure is used as fertilizer.

10. A campaign of education should be instituted in order that all who practise the home-canning of fruits and vegetables may be informed of the danger of infection with *Bacillus botulinus*.

BIBLIOGRAPHY.

1. Ueber ältere Beobachtungen, cf. Farcimen, *Mag. physiol. u. klin. Arzneimittellehre*, 1847, ii, 81; 1851, iii, 177, cited from Müller (81), xxi, 321.
2. Onolzbach, Actis physico-medico-forensibus colegii medici Onoldinicas, 1755, xxxi, cited from Kerner (7).
3. Cited from Kerner (7).
4. Cited from Müller (81), xxi, 322.
5. Jaeger, Nachricht und Warnung wegen des schädlichen Genusses geräucherter Blutwürste, Auftrage des Colegii archiatralis zu Stuttgart, *Reichsanzeiger*, 1802, No. 309, cited from Müller (81), xxi, 322.
6. von Autenrieth, J. F. H., in von Autenrieth, J. F. H., and von Bohnenberger, J. G. F., Tübinger Blätter für Naturwissenschaften und Arzneikunde, Tübingen, 1817, iii, 187, cited from Kerner (7).
7. Kerner, C. A. J., Neue Beobachtungen über die in Würtemberg so häufig vorfallenden tödtlichen Vergiftungen durch den Genuss geräucherter Würste, Tübingen, 1820.
8. Kerner, C. A. J., Das Fettgift, oder die Fettsäure, und ihre Wirkungen auf den thierischen Organismus. Ein Beytrag zur Untersuchung des in verdorbenen Würsten giftig wirkenden Stoffes, Stuttgart and Tübingen, 1822.
9. Weiss, Die neuesten Vergiftungen durch verdorbene Würste, beobachtet an neun und zwanzig Menschen in und um Murrhardt im Königreich Würtemberg, nebst der Versuche einer physiologisch-pathologischen Darstellung der Einwirkung dieses Giftes auf den Menschen, Karlsruhe, 1824.
10. Dann, E., De veneni botulini viribus et natura, Berolini, Inaugural dissertation, 1828, cited from Müller (81), xxi, 342.
11. Kahleis, Ueber das Wurstgift, *J. prakt. Heilk.*, 1821, liii, 44.
12. Publicandum der Regierung zu Arnsberg vom 18 Juni, 1822, Amstblatt, 1822, No. 25, cited from Senckpiehl (15), p. 13.
13. Schlossberger, J. E., Das Gift verdorbener Würste mit Berücksichtigung seiner Analogen in andern thierischen Nahrungsmitteln. 1ster Artikel: Historisch-kritischer Ueberblick der bisherigen Arbeiten und Ansichten, nebst dem Versuche einer neuen Theorie darüber, *Arch. physiol. Heilk.*, 1852, xi, 709.
14. von Faber, cited from Wosnitza (16).
15. Senckpiehl, P., Ueber Massenerkrankungen nach Fleischgenuss, besonders durch Wurst- und Fleischgift, Inaugural dissertation, Berlin, 1887.
16. Wosnitza, O., Beitrag zur Kasuistik des Botulismus, Inaugural dissertation, Leipsic, 1909.
17. Bürger, L., Ueber zwei Gruppen von Botulismus mit zwölf Erkrankungs- und fünf Todesfällen, *Med. Klin.*, 1913, ix, 1846.
18. Schumacher, E., Eine Gruppe von 6 klassischen Botulismuserkrankungen in der Eifel und der Nachweis ihres Erregers, des *Bacillus botulinus*, *Münch. med. Woch.*, 1913, lx, 124.

19. Paulus, E. A., Polioencephalomyelitis bei Botulismus, *J. Psychol. u. Neurol.*, 1915, xxi, 201.
20. Hoeg, N., Et Tilfælde af Botulisme, *Hospitalstid.*, 1915, lviii, 300.
21. Schede, Ueber Botulismus, *Med. Klin.*, 1916, xii, 1309, abstracted in *J. Am. Med. Assn.*, 1917, lxviii, 585.
22. Kaatzer, P., Ueber Vergiftung durch Wurstgift, *Deutsch. med. Woch.*, 1881, vii, 73.
23. Tripe, J. W., On poisoning by sausages, *Brit. and For. Med.-Chir. Rev.*, 1860, xxv, 142.
24. du Mesnil, O., Empoisonnement par de la viande de conserve, *Ann. hyg. pub.*, 1875, xliii, 472.
25. Mayer, Massenerkrankungen durch Nahrungs- und Genussmittel, *Deutsch. Vrtljschr. öff. Gsndhtspflg.*, 1913, xlv, 8.
26. von Horn, W., Dissertatio de veneno in botulis. Commentatio in certamine literario a gratioso medicorum ordine Berolinensi Praemio ornato, Berolini, 1828, abstracted in Arrowsmith, R., *Edinburgh Med. and Surg. J.*, 1830, xxxiii, 28.
27. Kerner, in Appendix, Weiss (9).
28. Hauff, Vergiftung durch geräuchertes Schweinefleisch, *J. prakt. Heilk.*, 1829, lxviii, 53.
29. Geiseler, E. F., *Rust's Mag.*, 1824, xvi, 111, cited from Schlossberger (13), p. 740.
30. Thorer, Vergiftung durch Schinken; Wurstgift, *Gen.-San.-Ber.* (Schlesien, year 1834), Breslau, 1837, 111, cited from Senckpiehl (15), p. 19.
31. Ulrich, R., Fünf Fälle von Fleischvergiftung in einer Familie, *Klin. Monatsbl. Augenh.*, 1882, xx, 235.
32. Roth, E., Zwei Fälle von Wurstvergiftung (Botulismus), *Vrtljschr. gerichtl. Med.*, 1883, xxxix, 241.
33. Groenouw, A., Fünf Fälle von Accommodationslähmung bei Fleischvergiftung (Schinken), *Klin. Monatsbl. Augenh.*, 1890, xxviii, 166.
34. van Ermengem, E., Contribution à l'étude des intoxications alimentaires, *Arch. pharmacod.*, 1897, iii, 213.
35. Römer, P., Ein Beitrag zur Aetiologie des Botulismus, *Centr. Bakteriol., Ite Abt.*, 1900, xxvii, 857.
36. Blattmann, Zur Casuistik des Botulismus, *Cor.-Bl. schweiz. Aerzte*, 1909, xxxix, 18.
37. Autenrieth, H. F., Ueber das Gift der Fische, Tübingen, 1833, cited from Schlossberger (13), p. 722; Grundriss der Sanitätspolizei von Dr. Nicolai, Berlin, 1835, 292, cited from Müller (81), xxi, 342.
38. Jaechnichen, *Med. Ztg. Russlands*, 1850, cited from Schlossberger (13), p. 741.
39. Schreiber, J., Ueber Fischvergiftung, *Berl. klin. Woch.*, 1884, xxi, 161.
40. Hirschfeld, 5 Fälle von Fischvergiftung mit 3 Todesfällen, *Vrtljschr. gerichtl. Med.*, 1885, xliii, 283.
41. von Anrep, V., L'intoxication par les ptomaïnes, *Arch. slaves biol.*, 1886, i, 341, abstracted in *Schmidt's Jahrb.*, 1887, ccxvi, 143.

42. Schmidt, N., Zur Frage über die Natur des Fischgiftes und dessen Wirkung auf den menschenlichen und thierischen Organismus, *Verhandl. x. internat. med. Cong.*, 1890, 4te Abt., ii, 43.
43. Arustamoff, M., Ueber die Natur des Fischgiftes, *Centr. Bakteriol.*, 1891, **x**, 113.
44. Tschernyschew, S., Ueber die pathologisch-anatomischen Veränderungen des Centralnervensystems welche durch Fischvergiftung erzeugt werden, *Ges. Neurol. u. Psychiat. in Moskau*, 19 Marz. Wratsch, xx, 449, abstracted in *Jahresb. Ophth.*, 1899, xxx, 433.
45. David, R., Botulismus nach Genuss verdorbener Fische, *Deutsch. med. Woch.*, 1899, xxv, 127.
46. Fischer, E., Drei Fälle von Ptomatropinvergiftung verursacht durch den Genuss von Krebsen, *St. Petersb. med. Woch.*, 1897, N. F. xiv, 472.
47. Böhm, R., Intoxicationen durch verdorbene Nahrungsmittel. Intoxication durch Fischgift, in von Ziemssen, H., Handbuch der speciellen Pathologie und Therapie, Leipsic, 1876, xv, 246.
48. Erben, F., in Dittrich, P., Handbuch der ärztliche Sachverständigentätigkeit, Vienna, 1910, vii, 1, 2te Hälfte, 840.
49. Esmein, C., Le botulisme, *Progrès. mêd.*, 1913, xliv, 575; *Presse mêd.*, 1914, xxii, 372.
50. Madsen, T., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, 1912, iv, cited from Ornstein (88), p. 467.
51. Siedler, *Mag. physiol. u. klin. Arzneimittellehre*, 1847, ii, 588, cited from Senckpiehl (15), p. 20.
52. Krugelstein, *Z. Staatsarzneik.*, 1839, xix, 261, cited from Müller (81), xxi, 343.
53. Homans, J., Partridge poisoning, *Boston Med. and Surg. J.*, 1871, lxxxv, 55, abstracted in *Jahresb. ges. Med.*, 1871, i, 367.
54. Cohn, H. L., Sehstörungen bei Vergiftungen durch Wildpastete und Hecht, *Arch. Augenh.*, 1879–80, ix, cited from Senckpiehl (15), p. 20.
55. Quincke, H., Ueber Fleischvergiftung, *Mitt. Ver. schlesw.-holst. Aerzte*, 1885, abstracted in *Jahresb. ges. Med.*, 1885, i, 569; abstracted in *Schmidt's Jahrb.*, 1887, ccxvi, 144.
56. Collatz, Vier Fälle von Botulismus, *Berl. klin. Woch.*, 1905, xlii (Fest-Nummer Carl Anton Ewald), No. 44*a*, 68.
57. Fischer, A., Ueber einen Massenerkrankung an Botulismus infolge Genuss "verdorbener" Bohnenkonserven, *Z. klin. Med.*, 1906, lix, 58.
58. Landmann, G., Ueber die Ursache der Darmstädter Bohnenvergiftung, *Hyg. Rundschau*, 1904, xiv, 449.
59. Wunderlich, cited from Weiss (9).
60. Cited from Müller (81).
61. Cited from Müller (81).
62. Emmert, C. F., Dissertatio inauguralis medica de venenatis acidi borussici in animalia effectibus, Tübigen, 1805, cited from Schlossberger (13), p. 730.

63. Kühn, C. G., Versuche und Beobachtungen über die Kleesäure, das Wurst- und das Käsegift, Leipsic, 1824.
64. Rühle, cited from Müller (81), xxi, 322.
65. Cormack and Corneliani, cited from Schlossberger (13), p. 730.
66. Jaeger, *Z. Staatsarzneik.*, 1823, vi, 471, cited from Müller (81), xxi, 341.
67. Buchner, *Z. Staatsarzneik.*, 1823, vi, 472, cited from Müller (81), xxi, 341; Toxikologie, 1st edition, cited from Schlossberger (13), p. 735.
68. Kastner, K. W. G., *Z. Staatsarzneik.*, 1823, vi, 470.
69. Bodenmüller, Ueber Wurstvergiftung, *Würt. Cor.-Bl.*, 1834, No. 38, abstracted in *Schmidt's Jahrb.*, 1835, v, 287.
70. Tritschler, *Würt. Cor.-Bl.*, xii, No. 13, cited from Müller (81), xxi, 343.
71. Lussana, *Omodei's Ann. med.*, Marzo, 1845, cited from Müller (81), xxi, 343.
72. Liebig, Die Chemie in ihrer Anwendung auf Agricultur und Physiologie, 5th edition, 1843, 472, cited from Schlossberger (13), p. 738.
73. Heller, *Arch. physiol. u. path. Chem. u. Micr.*, July, 1853, cited from Müller (81), xxi, 357.
74. van den Corput, Mémoire du poison qui se dévelopd dans les viandes, et les boudins fumés, Brussells, 1855, cited from Müller (81), xxi, 357.
75. Wittig, *Arch. Pharm.*, 1856, Vrtljschr. iv, cited from Kaatzer (22), p. 74.
76. Kasper, *Arch. Pharm.*, 1858, Vrtljschr. xiii, cited from Kaatzer (22), p. 74.
77. Zenker, F. A., Ueber die Trichinen-Krankheit des Menschen, *Arch. path. Anat.*, 1860, xviii, 561.
78. Virchow, Darstellung der Lehre von den Trichinen, 1864, cited from Kaatzer (22), p. 74.
79. Husemann, Toxicologie, Suppl.-Bd., 1866, cited from Kaatzer (22), p. 74.
80. Rupprecht, B., Die Trichinenkrankheit im Spiegel der Hettstädter Endemie betrachtet, 1864, cited from Müller (81), xxi, 358.
81. Müller, Das Wurstgift. (Ten chapters.), *Deutsch. Klin.*, 1869, xxi, 321; 1870, xxii, 27.
82. Ehrenberg, A., Ueber einige in einem Falle von sogenannter "Wurstvergiftung, aus dem schädlichen Materiale dargestellte Fäulnissbasen, sowie über einige, durch die Thätigkeit eines besonderen, in gleichen Materiale aufgefundenen, Bacillus gebildete Zersetzungs-Producte, *Z. physiol. Chem.*, 1887, xi, 239.
83. Nauwerck, C., Ueber Wurstvergiftung, *Münch. med. Woch.*, 1886, xxxiii, 538.
84. Redner, cited from Nauwerck (83), p. 538.
85. van Ermengem, E., Untersuchungen über Fälle von Fleischvergiftung mit Symptomen von Botulismus, *Centr. Bakteriol., Ite Abt.*, 1896, xix, 442.
86. van Ermengem, E., Ueber einen neuen anaëroben Bacillus und seine Beziehungen zum Botulismus, *Z. Hyg. u. Infektionskrankh.*, 1897, xxvi, 1.
87. van Ermengem, E., Der *Bacillus botulinus* und der Botulismus, in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1912, iv, 909.

88. Ornstein, O., Ein Fall von Botulismus, *Z. Chemotherap., Orig.*, 1913, i, 458.
89. Seelye, H. H., Atropia-poisoning from eating turkey, *Med. Rec.*, 1894, xlv, 14.
90. Herzog, A. W., Turkey and ptomaine, *Med. Rec.*, 1894, xlv, 155.
91. Spiller, W. G., Neuritis from the ingestion of putrefying pork, *Phil. Polyclin.*, 1898, vii, 455.
92. Lewis, W. M., A case of ptomaine poisoning, *South. Calif. Pract.*, 1899, xiv, 464.
93. Jellinek, E. O., On ptomaine poisoning, *Calif. State J. Med.*, 1902–03, i, 121; A report of three cases of ptomaine poisoning, *Pacific Med. J.*, 1903, xlvi, 110.
94. Anderson, M., Ptomain poisoning; a case, *Woman's Med. J.*, 1904, xiv, 153.
95. Bryant, W. S., Deaf-mutism and ptomaine poisoning, *Tr. Am. Otol. Soc.*, 1905, ix, 32.
96. Sheppard, C., Report of three cases of fatal ptomaine poisoning, *South. Calif. Pract.*, 1907, xxii, 370.
97. Peck, G. W., Ptomaine poisoning; report of eleven fatal cases at Sawtelle, California, *South. Calif. Pract.*, 1910, xxv, 121.
98. Stiles, P. G., A case of apparent food-poisoning of the type known as botulism or allantiasis, *J. Am. Med. Assn.*, 1913, lxi, 2301.
99. Wilbur, R. L., and Ophüls, W., Botulism. A report of food-poisoning apparently due to eating of canned string beans, with pathological report of a fatal case, *Arch. Int. Med.*, 1914, xiv, 589.
100. Williams, T. M., personal communication.
101. Frost, L. C., Meat poisoning with report of cases, *Am. Med.*, 1915, N. S. x, 85.
102. Lancaster, W. B., A case of botulism, *Tr. Am. Ophth. Soc.*, 1916, xiv, 648; *Ophthalmoscope*, 1916, xiv, 588.
103. Curfman, G. H., Botulism, *Colorado Med.*, 1917, xiv, 35.
104. Lebert, cited from Müller (81), xxi, 381.
105. Ruge, S., Ein Fall von Papilloretinitis bei Botulismus, *Klin. Monatsbl. Augenh.*, 1902, xl, 408.
106. Niedner, Ein Fall von Wurstvergiftung, *Berl. klin. Woch.*, 1866, iii, 2.
107. Wertheim, E., Ein Fall von Velumlähmung infolge von Botulismus, *Arch. Laryngol. u. Rhinol.*, 1902–03, xiii, 454.
108. Eichenberg, F., Ueber Vergiftung durch Wurstgift, Inaugural dissertation, Göttingen, 1880, abstracted in *Schmidt's Jahrb.*, 1881, cxci, 8.
109. Fischer, cited from Erben (48), p. 849.
110. Lochte, Die Amtsärztliche Beurtheilung der Fleischvergiftung (Botulismus), *Deutsch. Vrtljschr. öff. Gsndhtspflg.*, abstracted in *Jahresb. ges. Med.*, 1903, i, 591.
111. Novy, F. G., Food poisons, in Osler, W., and McCrae, T., Modern medicine, New York, 2nd edition, 1914, ii, 450.
112. Konstansoff, S. V., cited from Novy (111), p. 453.
113. Gaffky, G. J. A., cited from Leuchs (114).

114. Leuchs, J., Beiträge zur Kentniss des Toxins und Antitoxins des *Bacillus botulinus*, *Z. Hyg.*, 1910, lxv, 55.
115. Kempner, W., and Pollack, B., Die Wirkung des Botulismustoxins (Fleischgiftes) und seines specifischen Antitoxins auf die Nervenzellen, *Deutsch. med. Woch.*, 1897, xxiii, 505.
116. von Hibler, E., in Fischer, G., Untersuchungen über die pathogenen Anaëroben, Jena, 1908.
117. Harrass, P., Zur Frage der aëroben Züchtung sogenannter obligat-anaërober Bakterien, *Münch. med. Woch.*, 1906, liii, 2237.
118. Tarozzi, G., Ueber ein leicht in aërober Weise ausführbares Kulturmittel von einigen bis jetzt für strenge Anaëroben gehaltenen Keimen, *Centr. Bakteriol., 1te Abt., Orig.*, 1905, xxxviii, 619.
119. Forssman, J., *Centr. Bakteriol.*, 1901, xxix, cited from van Ermengem (87), p. 923.
120. Vaillard, L., and Rouget, J., Note au sujet de l'étiologie du tétanos, *Ann. Inst. Pasteur*, 1893, vii, 755.
121. Pelzl, O., Ueber Botulismus, *Wien. klin. Woch.*, 1904, xvii, 864.
122. Brieger and Kempner, W., Beitrag zur Lehre von der Fleischvergiftung, *Deutsch. med. Woch.*, 1897, xxiii, 521.
123. Kempner, W., and Schepilewsky, E., Ueber antitoxische Substanzen gegenüber dem Botulismusgift, *Z. Hyg.*, 1898, xxvii, 213.
124. Lippmann, H., Ueber lokale Immunisierung der Eingangspforten von Infektionen, *Med. Klin.*, 1910, vi, 1477, abstracted in *Centr. Bakteriol., 1te Abt., Ref.*, 1911, xlix, 549.
125. Forssman, J., Beiträge zur Kenntnis der Bakteriologie des Botulismus (Lunds Universitets Årsskrift, 1900), abstracted in *Centr. Bakteriol., 1te Abt.*, 1901, xxix, 541.
126. Roux and Borrel, abstracted in *Centr. Bakteriol.*, cited from Forssman (125), p. 541.
127. Kob, M., Beitrag zur Kenntnis des Botulismus, *Med. Klin.*, 1905, i, 84.
128. Kempner, W., Weiterer Beitrag zur Lehre von der Fleischvergiftung. Das Antitoxin des Botulismus, *Z. Hyg. u. Infektionskrankh.*, 1897, xxvi, 481.
129. Forssman, J., and Lundstrom, E., Sur la marche de la courbe d'antitoxine dans l'immunisation active contre le botulisme, *Ann. Inst. Pasteur*, 1902, xvi, 294.
130. Forssman, J., Studien über die Antitoxinbildung bei aktiver Immunisierung gegen Botulismus, *Centr. Bakteriol., 1te Abt., Orig.*, 1905, xxxviii, 463.
131. Tchitchkine, A., Essai d'immunisation par la voie gastro-intestinale contre la toxine botulique, *Ann. Inst. Pasteur*, 1905, xix, 335.
132. Herman, Serumdiagnose bei Fleischvergiftungen, Zehnter internationaler Kongress für Hygiene und Dermographie zu Paris, 1900, *Deutsch. Vrtljschr. öff. Gsndhtspflg.*, 1900, xxxii, 710, abstracted in *Centr. Bakteriol., 1te Abt.*, 1901, xxix, 706.

133. Leuchs, J., *Bacillus botulinus.* (Immunität.), in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1912, iv, 939.
134. van Hasselt, A. W. M., in Henkel, J. B., Handbuch der Giftlehre, Braunschweig, 1862, pt. 2, 146.
135. van der Stricht, O., Nouvelles recherches sur la genèse des corpuscules rouges et des globules blancs du sang, *Arch. Biol.*, 1892, xii, 199, cited from van Ermengem (86), p. 48.
136. Marinesco, G., Pathologie générale de la cellule nerveuse; lésions secondaires et primitives, *Presse mêd.*, 1897, v, 41.
137. Ossipoff, V. P., Influence de l'intoxication botulinique sur le système nerveux central, *Ann. Inst. Pasteur*, 1900, xiv, 769.
138. Römer, P., and Stein, L., Experimenteller Beitrag zur Frage nach dem Sitz und Wesen der Accomodationsparese bei bakteriellen Intoxikationskrankheiten. Die Accomodationsparese, *Arch. Ophth.*, 1904, lviii, 291.
139. Komotzki, W., Experimentelle Untersuchungen über die Wirkung des Botulismus-Toxins auf die inneren Organe, *Virchows Arch. path. Anat.*, 1911, ccvi, 179.
140. Bogomolez, A., Ueber die Hypersekretion von Lipoidsubstanz durch die Rinde der Nebennieren bei experimentellem Botulismus, *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 35.
141. Dickson, E. C., Botulism, an experimental study. A preliminary report, *J. Am. Med. Assn.*, 1915, lxv, 492.
142. Kiyono, K., Die vitale Karminspeicherung, Jena, 1904.
143. Goldmann, E. E., Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung," *Beitr. klin. Chir.*, 1909, lxiv, 192.
144. MacCurdy, J. T., and Evans, H. M., Experimentelle Läsionen der Centralnervensystems, untersucht mit Hilfe der vitalen Färbung, *Berl. klin. Woch.*, 1912, xlix, 1695.
145. Pürckhauer, H., Zur Casuistik der Allantiasis, *Aerztl. Int.-Bl.*, 1877, xxiv, 245.
146. Wolffhügel and Hueppe, Ueber das Eindringen der Hitze in das Fleisch bei seiner Zubereitung, *Mitt. k. Gsndhtsamte.*, 1881, i, 395.
147. Saunders, E. W., The green fly (*Lucilia Cæsar*) as the universal destroyer of motor function and of life, *J. Arkansas Med. Soc.*, 1915–16, xii, 6. Saunders, E. W., Meisenbach, R., and Wisdom, W. E., The causation and prevention of infant paralysis, *J. Missouri Med. Assn.*, 1913–14, x, 305.
148. Buckley, J. S., and Shippen, L. P., Preliminary report on the relation of anaerobic organisms to forage poisoning, *J. Am. Vet. Med. Assn.*, 1917, l, 809.
149. Wassermann, cited from Leuchs (133).
150. Schneidemühl, Ueber Botulismus beim Menschen und die sogenannte Geburtsparalyse bei Rindern, *Centr. Bakteriol., 1te Abt.*, 1898, xxiv, 577.
151. Bail, O., Versuche über eine Möglichkeit der Entstehung von Fleischvergiftungen, *Hyg. Rundschau*, 1900, x, 1017.

References Which Have Not Been Cited.

1. Christison, R., A treatise of poisons, in relation to medical jurisprudence, physiology, and the practice of physic, Edinburgh, 2nd edition, 1832, 557.
2. Paulus, Neue Beiträge zur Geschichte der Vergiftung durch verdorbene Wurstmasse, *Heidelb. Ann.*, 1834, x, Heft. 3, abstracted in *Schmidt's Jahrb.*, 1835, vi, 22.
3. von Faber, Ueber Wurstvergiftungen, *Würt. Cor.-Bl.*, 1854, No. 33, abstracted in *Schmidt's Jahrb.*, 1855, lxxxv, 36.
4. Boehm, R., Wurstvergiftung, Botulismus, Allantiasis, in von Ziemssen, H., Handbuch der speciellen Pathologie und Therapie, 1876, xv, 235; Translation, *Cyclop. Pract. Med. N. Y.*, 1878, xvii, 535.
5. Reisz, P., Sieben Fälle von Wurstvergiftung (Botulismus), *Wien. med. Presse*, 1891, xxxii, 1862. (Report of the use of eserine and pilocarpin.)
6. Brosch, A., Zur Casuistik der Fischvergiftung, *Wien. klin. Woch.*, 1896, ix, 219. (Report of poisoning from oysters. Symptoms similar to those of botulism.)
7. Dieudonné, A., Bacterial food poisoning, translation by Bolduan, New York, 1909, 62.
8. Dickson, E. C., Botulism, its occurrence in California, *Calif. State J. Med.*, 1916, xiv, 143.
9. Dickson, E. C., Botulism, *Proc. Soc. Exp. Biol. and Med.*, 1916, xiv, 47.
10. Dickson, E. C., Botulism. A cause of limber-neck in chickens, *J. Am. Vet. Med. Assn.*, 1917, l, 612.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. *Bacillus botulinus*. The film is from a slant agar culture which had grown for 1 week. Note the bacilli without spores, the club-shaped and spindle-shaped, spore-bearing bacilli, and the numerous spores.

FIG. 2. R. L., Stanford University Series. This is one of the thrombi in the case which was described by Ophüls (99), and is in a vein of the cerebral meninges.

The patient died 13 days after she had eaten the string bean salad.

PLATE 2.

FIG. 3. Mrs. R., Outbreak 4 of my series. The thrombus is in a vein of the cerebral meninges. Note the accumulation of leukocytes.

The patient died 5 days after she had tasted the string beans.

FIG. 4. Mrs. R. The thrombus is in an artery in the lungs. Note the accumulation of leukocytes at one end of the thrombus. Adjacent is a bronchus which shows purulent bronchitis. There was a marked bronchopneumonia.

PLATE 3.

FIG. 5. Mrs. M. P., Case 2, Outbreak 7 of my series. The thrombus is in a vein in the cerebral meninges. Note the small number of leukocytes.

The patient died 7 days after she had eaten the string bean salad.

FIG. 6. Mrs. M. P. The thrombus is in a vein in a trabecula of the spleen. Note the absence of leukocytes.

PLATE 4.

FIG. 7. Rabbit 13, Series 1914–15. The hemorrhages are in the tissue of the brain stem in the region of the fourth ventricle, and near the origin of the peduncle. There were typical thrombi in the blood vessels.

The animal received 5 cc., intraperitoneal injection, of fluid from can of string beans, No. 10, which had been inoculated from Strain I, 12 months previously.

FIG. 8. Guinea Pig 29, Series 1916–17. The hemorrhage is in the lung, and in addition to the large area there are many red blood corpuscles scattered through the adjacent air spaces.

The animal received 2 cc., subcutaneous injection, of an apricot stone infusion culture of Strain III. It died in about 24 hours after the injection.

PLATE 5.

FIG. 9. Cat 3, Series 1914–15. The thrombus is shown under high power, and is in a vein in one of the deep sulci of the cerebrum. Note the thick bands of fibrin and the dense accumulation of leukocytes.

The animal received 2 cc., intraperitoneal injection, of the fluid from can of string beans, No. 6, which had been inoculated with Strain I, 3 months previously.

FIG. 10. Rabbit 35, Series 1915–16. The thrombus is in a vein in the cerebral meninges. Note the accumulation of leukocytes and the masses of conglutinated blood platelets.

The animal received 3.5 cc., subcutaneous and intraperitoneal injections, of a 2 month beef infusion culture of Strain II. It died 5 days after the injection.

PLATE 6.

FIG. 11. Rabbit 15, Series 1914–15. The thrombus is in a vein of the cerebral meninges. Note the clumping of the leukocytes and the whorled arrangement of the fibrin in the center of the vein.

The animal received 5 cc., intraperitoneal and subcutaneous injections, of fluid from can of string beans, No. 12, which had been inoculated with Strain I 12 months previously.

FIG. 12. Cat 31, Series 1916–17. The thrombus is in the central vein of a liver lobule.

The animal received 1 cc., subcutaneous injection, of a beef infusion culture of Strain III.

PLATE 7.

FIG. 13. Cat 29, Series 1916–17. Note the parenchymatous degeneration of the epithelium of the kidney tubules and the exudate in the clefts of Bowman's capsules. Some of the glomerular clefts contained red blood corpuscles.

The animal received 4 cc., subcutaneous injection, of a 25 day culture of Strain III in beef infusion. It died 7 days after the injection.

FIG. 14, Cat 12, Series 1914–15. The thrombi are in veins and an artery in the space between the lower surface of the posterior lobe of the cerebrum and the upper surface of the stem. Note the dense accumulations of leukocytes.

The animal received 5 cc., subcutaneous and intraperitoneal injection, of fluid from can of string beans, No. 12, which had been inoculated with Strain I, 12 months previously.

PLATE 8.

FIG. 15. Cat 14, Series 1915–16. The thrombus is in a vein of the cerebellar meninges. Note the dense accumulation of leukocytes.

The animal received 4 cc., intraperitoneal injection, of a 14 day bean infusion culture of Strain I. It died 4 weeks after the injection.

FIG. 16. Cat 20, Series 1916–17. The thrombus is in a vein in the meninges of the cord. Note the small mass of thrombus with thick bands of fibrin and accumulation of leukocytes, and also the loose fibrin in the outer portion of the vein.

The animal received 0.5 cc., subcutaneous injection, of a pea infusion culture of Strain III. It died 2 days after the injection.

PLATE 9.

FIG. 17. Cat 25, Series 1915–16. The thrombus is in a vein in the cerebral meninges. Note the accumulation of leukocytes.

The animal received 5 cc., intraperitoneal injection, of a 5 week beef infusion culture of Strain I. It died 16 days later.

FIG. 18. Guinea Pig 33, Series 1915–16. The thrombus is in one of the posterior cerebral arteries. Note the absence of leukocytes.

The animal received 5 cc., intraperitoneal injection, of a 6 day culture of Strain I in beef infusion.

PLATE 10.

FIG. 19. Rabbit 35, Series 1915–16. The thrombus is in an artery of the cerebral meninges. Note the relatively few leukocytes, and the small round mass of conglutinated blood platelets at one end of the thrombus.

The animal received 3.5 cc., subcutaneous injection, of a 2 month beef infusion culture of Strain II. It died 5 days after the injection.

FIG. 20. Cat 35, Series 1916–17. The thrombus is in an artery in the cerebral meninges. Note the relatively small number of leukocytes and the absence of dense bands of fibrin.

The animal received 0.004 cc., subcutaneous injection, of a beef infusion culture of Strain IV. It died 3 days after the injection.

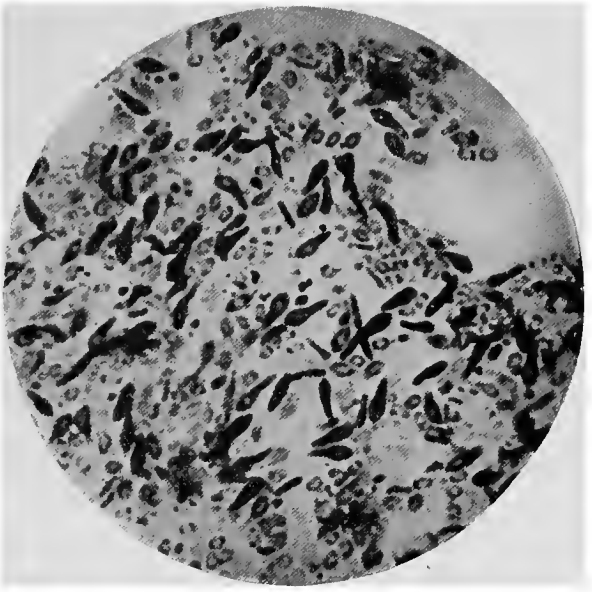


FIG. 1.

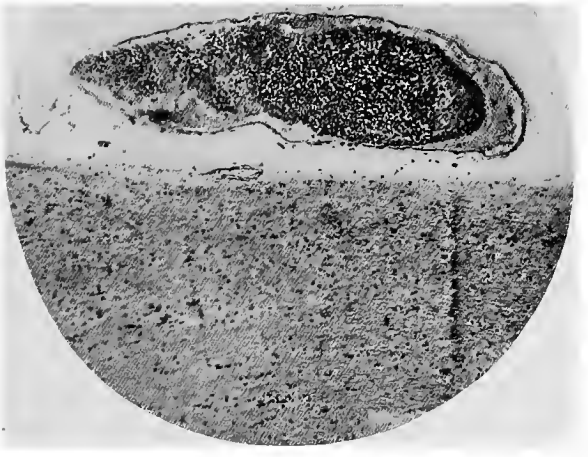


FIG. 2.

(Dickson: Botulism.)

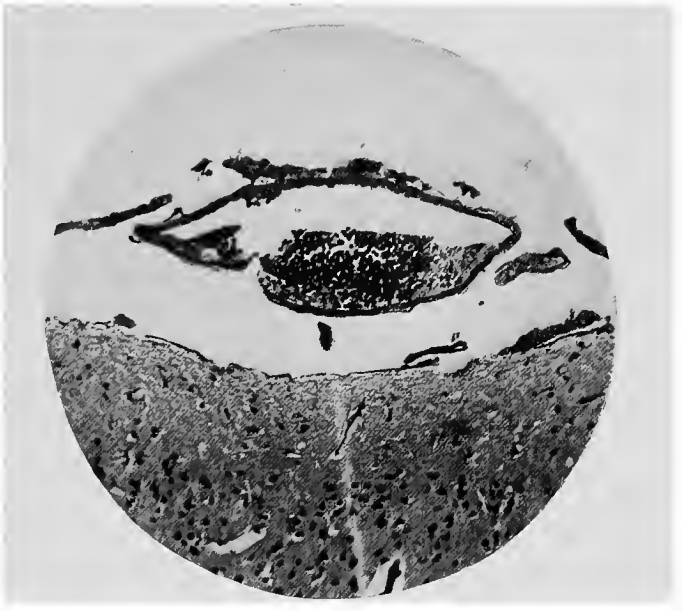


FIG. 3.

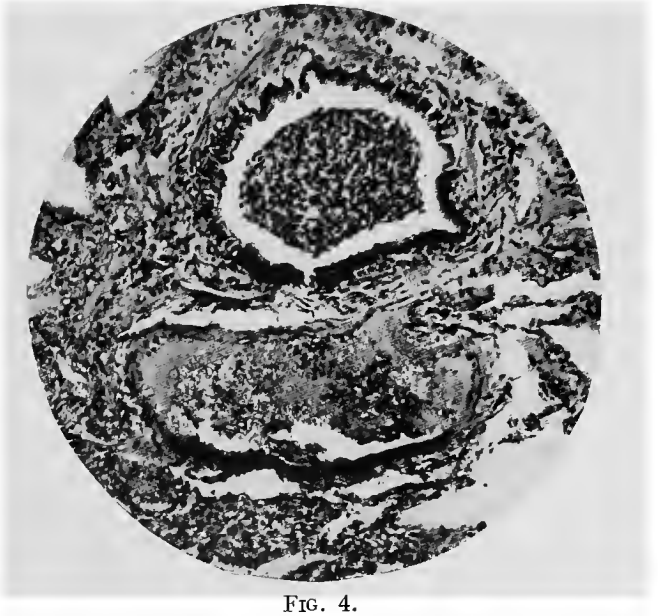


FIG. 4.

(Dickson: Botulism.)

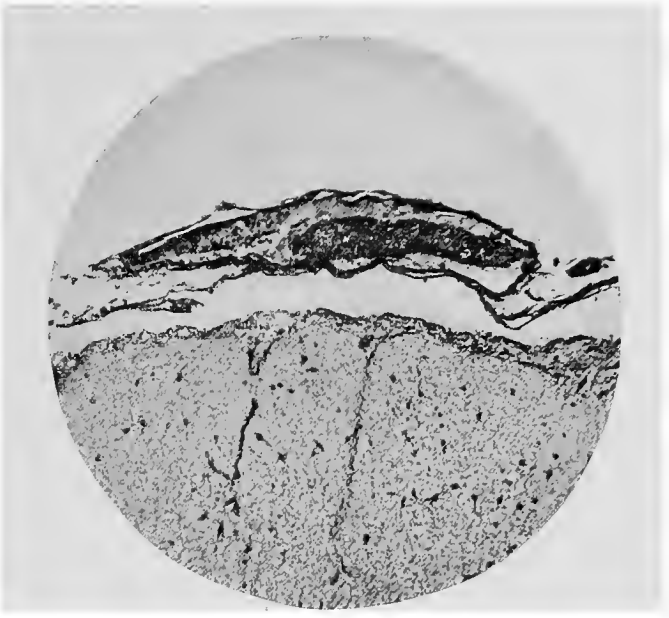


FIG. 5.

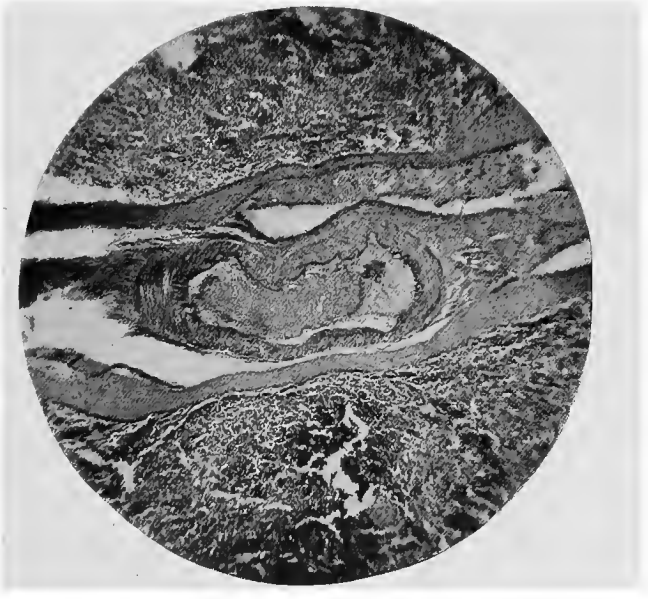


FIG. 6.

(Dickson: Botulism.)

FIG. 7.

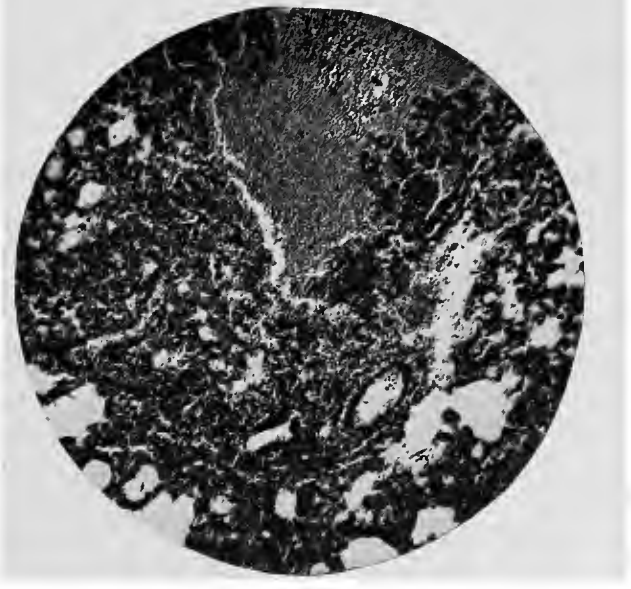


FIG. 8.

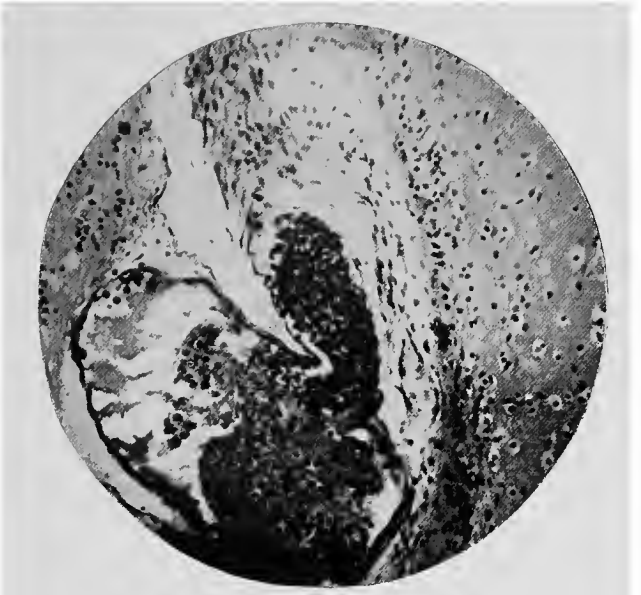


FIG. 9.

FIG. 10.

(Dickson: Botulism.)

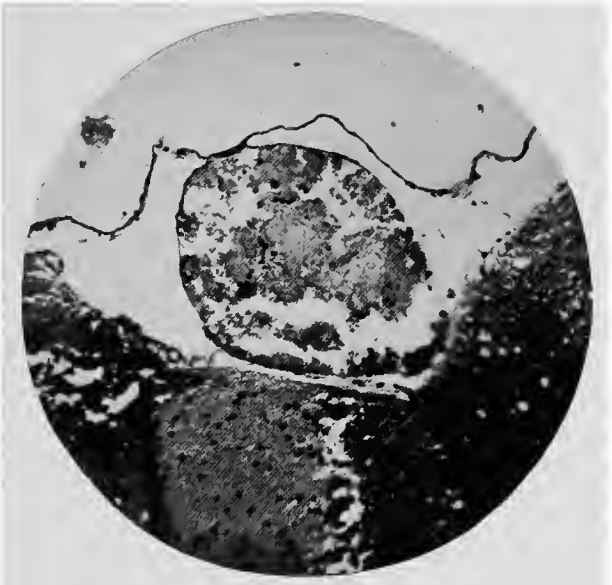


FIG. 11.

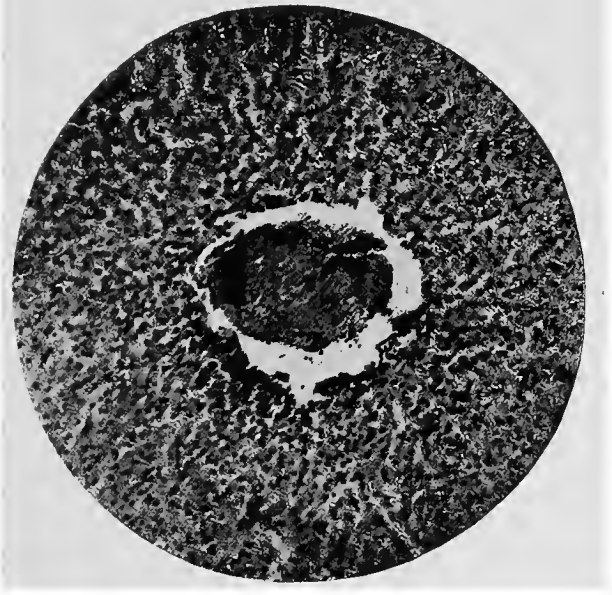


FIG. 12.

(Dickson: Botulism.)

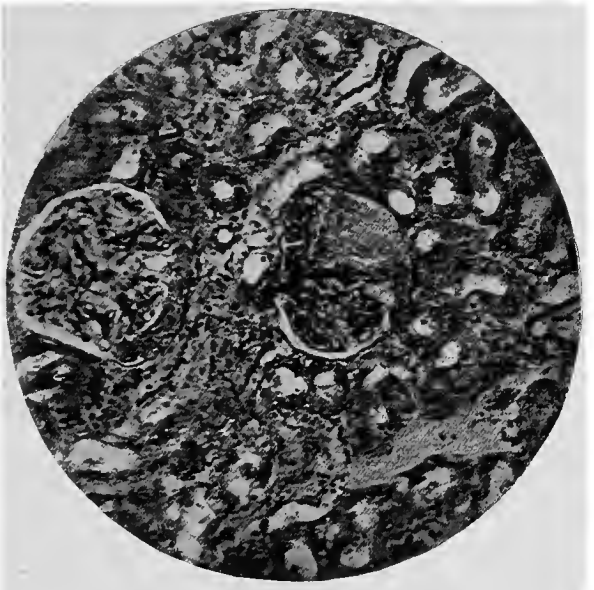


FIG. 13.

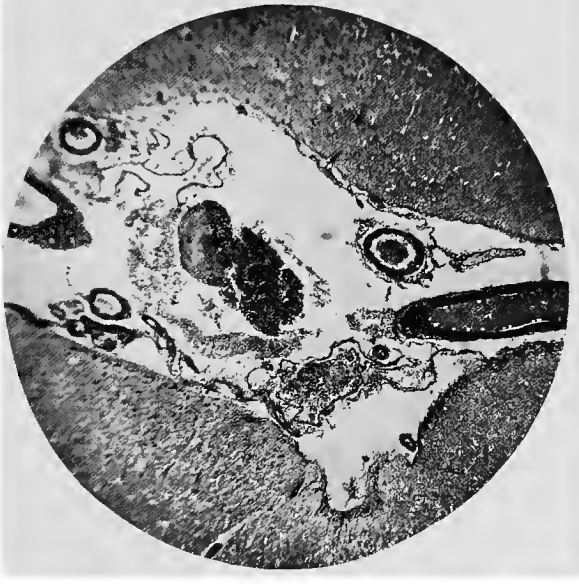


FIG. 14.

(Dickson: Botulism.)

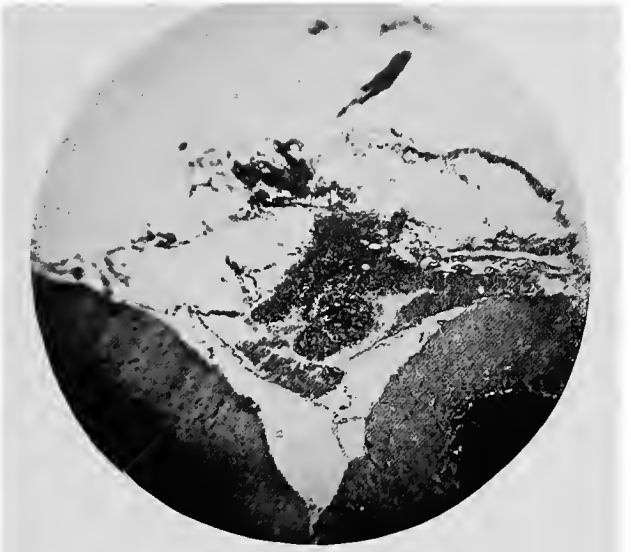


FIG. 15.

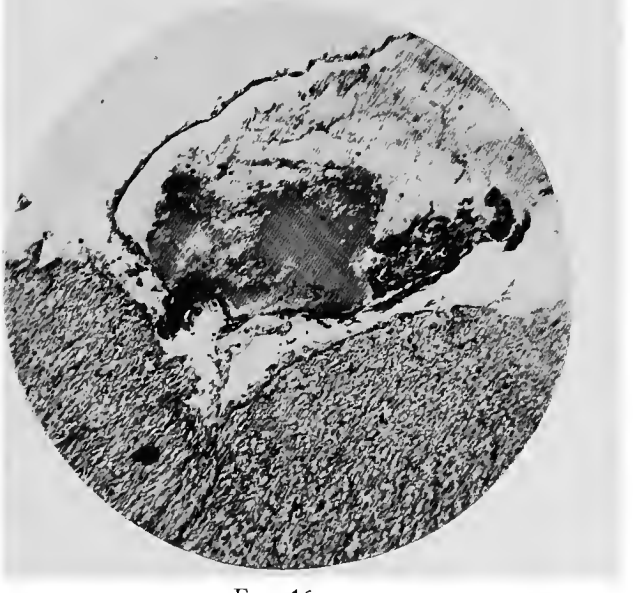


FIG. 16.

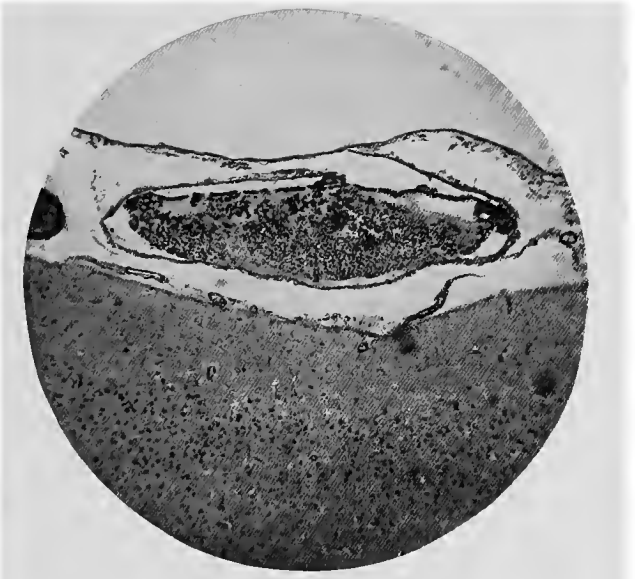


FIG. 17.

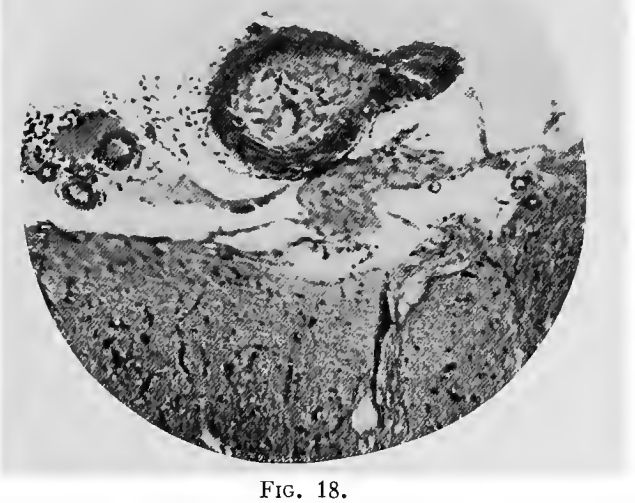


FIG. 18.

(Dickson: Botulism.)

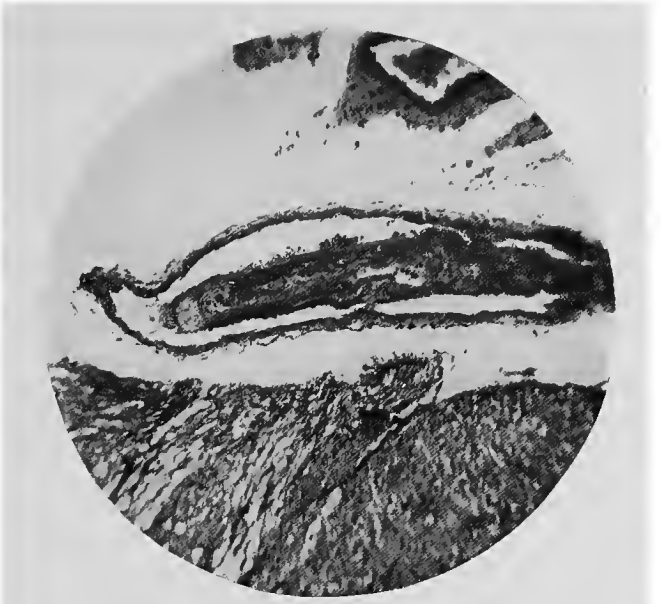


FIG. 19.

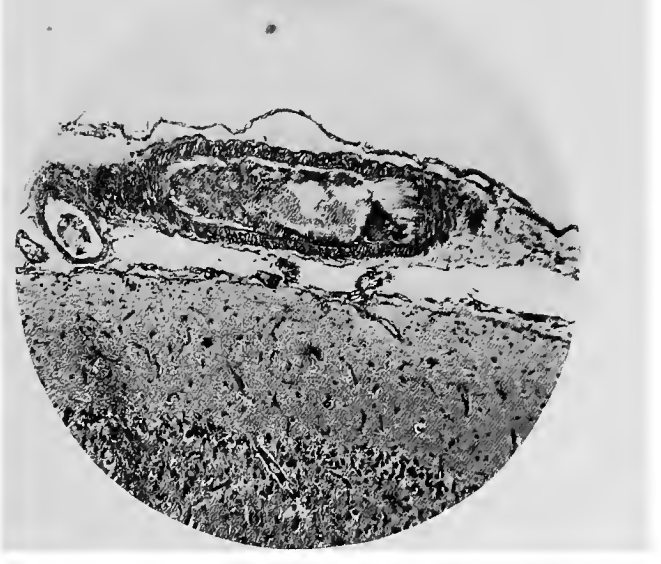


FIG. 20.

MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Under the head of *Monographs of The Rockefeller Institute for Medical Research* are published from time to time scientific papers which are so extensive, or require such elaborate illustrations, as to render them unsuitable for current periodical issues. The *Monographs* are published at irregular periods, determined by the available material on hand. The publication of new monographs is advertised on the cover of *The Journal of Experimental Medicine.*

Monograph No. 1.

(Issued June 30, 1910.)

SIMON FLEXNER and J. W. JOBLING. Studies upon a transplantable rat tumor. Plates 1–16. J. W. JOBLING. The biology of a mixed tumor of the rat. MAUD L. MENTEN. Experiments on the influence of radium bromide on a carcinomatous tumor of the rat. J. W. JOBLING. Spontaneous tumors of the mouse. Plates 17–28. J. W. JOBLING. Transplantation experiments in *Macacus rhesus* with a carcinomatous teratoma from man.

Monograph No. 2.

(Issued February 15, 1911.)

WILLIAM TRAVIS HOWARD and OSCAR T. SCHULTZ. Studies in the biology of tumor cells. Plates 1–6.

Monograph No. 3.

(Issued March 15, 1911.)

BENJAMIN J. TERRY. Chemo-therapeutic trypanosome studies with special reference to the immunity following cure.

Monograph No. 4.

(Issued June 24, 1912.)

FRANCIS W. PEABODY, GEORGE DRAPER, and A. R. DOCHEZ. A clinical study of acute poliomyelitis. Plates 1–13.

Monograph No. 5.

(Issued September 27, 1915.)

J. P. SIMONDS. Studies in *Bacillus welchii*, with special reference to classification and to its relation to diarrhea.

Monograph No. 6.

(Issued January 31, 1916.)

JAMES L. STODDARD and ELLIOTT C. CUTLER. Torula infection in man. Plates 1–9.

Monograph No. 7.

(Issued October 16, 1917.)

OSWALD T. AVERY, H. T. CHICKERING, RUFUS COLE, and A. R. DOCHEZ. Acute lobar pneumonia. Prevention and serum treatment. Plates 1–3.

Monograph No. 8.

(Issued July 31, 1918.)

ERNEST C. DICKSON. Botulism. A clinical and experimental study. Plates 1–10.

Monograph No. 9.

(In press.)

JAMES HOWARD BROWN. The use of blood agar for the study of streptococci. Plates 1-36.

Monographs will be sent postpaid on application, at $1.00 each; payable in advance by check or money order.

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

The Journal of Experimental Medicine is published under the auspices of The Rockefeller Institute for Medical Research and is designed to cover the field of experimental medicine. It is a medium for the publication of investigations conducted at the Institute, or elsewhere under its grants; and it also accepts contributions of a suitable character from other sources.

Contributions should be addressed to the Editor of *The Journal of Experimental Medicine*, 66th Street and Avenue A, New York. They should invariably be written in the English language, and limited preferably to twenty printed pages, not counting the space occupied by illustrations. Articles which exceed in length twenty-five printed pages will be returned to the authors in order that their contents may be reduced to this maximum. Authors receive fifty reprints of their papers free of charge; additional copies may be obtained at cost.

The Journal of Experimental Medicine is published monthly, the issues of each calendar year making two volumes.

The subscription price per year (two volumes) is $5.00. Single numbers cost 75 cents.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

H. D. DAKIN, New York, N. Y. — A. N. RICHARDS, Philadelphia, Pa.
E. K. DUNHAM, New York, N. Y. — DONALD D. VAN SLYKE, New York, N. Y.
L. B. MENDEL, New Haven, Conn. — CLARENCE J. WEST, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of between five and six hundred pages. The price of *The Journal* to subscribers in the United States and Canada is $3.00 per volume; in other countries, $3.25. Contributors receive 100 copies of their papers free of charge; additional copies are furnished at cost.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research.* Price, $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine* or *The Journal of Biological Chemistry.*

MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

For information regarding the *Monographs* see the inside of this cover.

All inquiries, correspondence, and subscriptions should be addressed to the

PUBLICATION DEPARTMENT
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
66TH STREET AND AVENUE A, NEW YORK