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THE PATHOGENESIS OF BOTULISM

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INTRODUCTION

The science of botulism was born in the pre-bacteriological period. The first detailed clinical-epidemological description of this illness was made by Zengbush in Russia (1818) and by the Wuert-temberg poet and physician Justinius Kerner (1820). Thanks to their experimental research and clinical observation, there was established a causal relationship between the consumption in food of old sausage products, red fish and the subsequent illness with a characteristic symptomatology and a clinical chart. The designation of this illness with the name "botulism" derives from the Latin word "botulus" - meaning sausage.

The pathological condition, which develops with botulism in the human being, was considered to be a chemical poisoning from poisonous substances that had formed in spoiled sausage and fish. In order to explain the reasons for the poisoning, ~~ik~~ various theories were propounded. Certain authors (Kerner, Weiss, Ruele and others) claimed that the source of poisoning from sausage was a fatty acid ("corpse acid"); in the opinion of others (Emmert and Kuen), prussic acid; and finally, some persons considered that sausage poison was a ferment (Libich) or a volatile base from the group of alkaloids (Schlossberger).

The development of organic chemistry in the 70-ties and 80-ties of the last century brought a reverberation in the establishment of the ptomaine theory of botulism. In accordance with this theory, the pathogenesis of botulism was explained as the poisoning of the system by toxic substances (ptomaines) which form in food products (sausage, ham, meat, fish and others), providing these have undergone decomposition under the apropriate temperature conditions.. All of

these contradictory theories about chemical poisons, precipitating either sausage or fish poisoning, have been shown to be absolutely unsound. Over the course of the nineteenth century, the reason why the poison was formed and the very nature of the poison remained a complete riddle.

In 1896, Van Ermeng discovered the stimulant of botulism. He was able to separate it from the remains of ham and also from the spleen and the fat intestines of a man who had died due to poisoning from this ham.

The discovered stimulant received the name Bac. Botulinus. Van Ermeng's discovery soon was supported by many authors on various pages. An especially valuable observation was made by the Russian researcher Konstansov (1904) who isolated the stimulant from fish that had caused "fish poisoning." The study of this microbe was shown to be completely identical with the Bac. Botulinus of Van Ermeng.

As a basis for his experimental research, Van Ermeng created a toxic theory of pathological condition for botulism. According to this theory, the stimulant of botulism - toxigenic saprophyte - does not have the ability to propagate itself in the organism of a human being or an animal; and pathological phenomena are precipitated only by the toxin which is in turn formed inside of the organism in food products or in fodder. This purely toxic theory, which excludes the role of the microbe in the development of the pathological process, represents an echo of the chemical (ptomaine) theory of botulism.

Regardless of its far-reaching errors, the theory of Van Ermeng nevertheless played a pioneering part in science. In caused

researchers to cease paying attention to chemical poisons, as the alleged cause for the pathological phenomena in botulism, and to concentrate on the microbe factor.

It was necessary to accumulate tremendous material of an experimental and clinical-epidemological nature in order to establish the erroniousness of Van Ermeng's toxic theory, which for over 30 years had occupied the leading position in medicine. It is sufficient to point out that to this time, as if this were not surprising, many researchers continue to remain on the positions of the toxic theory.

Contemporary substantiated data of a pathogenetic and clinical-epidemological nature conclusively refute the toxic theory for the pathogenesis of botulism, and they are the foundation for a rational theory on the toxic-infectious nature of botulism.

The study about the pathogenesis of botulism in the human being represents one of the most important parts of the problem of botulism in general. Without a scientifically substantiated representation of this matter, it would be impossible to find the correct theoretical and practical solution to the whole problem.

That is why we have set ourselves the task of presenting in the following monograph an exposition in systematical sequence of this prolific material from the study of the pathogenesis of botulism, which material was collected in contemporary times by Soviet and foreign researchers.

However, the basic attention in the compilation of these materials was given to experimental research, which was conducted by us over 12 years on the study of infectious properties of the stimulant of botulism in the human being and in animals.

The data obtained from the bacteriological, immunological and physiological experiments conducted by us as well as the material from numerous Soviet authors conclusively substantiate the toxic-infectious nature of botulism in the human being.

At the current time, the problem still remains unsolved as to whether food products that have been relieved of toxin, but which contain spores of botulism baccilli, can cause botulism in a human being. If it can be accepted as established that large quantities of spores cause botulism in animals, then certainly the fate of small quantities of spores is unknown even when these penetrate into the organism of the human being during the consumption of food infected by the stimulant of botulism.

In view of the fact that this mocrobe is quite widely distributed in nature and that many products are infected with it, and even fruit is not rarely contaminated, the study of this problem represents itself of great theoretical and practical interest.

Research on the pathogenesis of a botulism infection has brought us to the necessity of a more thorough study of the pathogenesis of botulism intoxication and certain questions involving immunity to such poisoning, which appear to be pfoblems never studied.

As the pathogenetic process developing in the organism during botulism creates the conditions for the formation and development of reactions and changes of an immunological nature, it has appeared necessary to introduce into this monograph a chapter in which the most substantive data on immunity to botulism are given.

A considerable amount of attention has been provided in this monograph to the condition of animal organisms during their infection with sub-lethal doses of spores from botulism bacilli. The results

of the conducted experimental research can, in our opinion, hasten the explanation of problems pertaining to the pathogenesis and the immunity from other infectious diseases (tetanus, gaseous gangrene).

Realizing all of the difficulties in the task he has set himself, the author in advance transmits his thanks for calling his attention to possible defects and ommissions in this work.

C H A P T E R I

BRIEF DESCRIPTION OF THE BIOLOGICAL PROPERTIES PERTAINING TO THE STIMULANT FOR BOTULISM

The stimulant for botulism, Bac. Botulinus (Clostridium Botulinum according to the American classification), represents a bacillus having rounded ends which is on the average from 5 to 10μ in length and from 0.3 to 0.8 μ in width. Thanks to the braids, which are situated on its whole body, the bacillus is provided with mobility. In the soil, in food products, and in nourishing surroundings it forms spores. This microbe appears to be a very rigid anaerobe and very sensitive to oxygen. In difficult alimentary surroundings, it is possible to obtain a surface growth while providing not more than 15 millimeters of a mercury column.

The bacilli of botulism have been traditionally divided into five types, differing among one another in agglutinations and in the antigenic properties of toxins. At the present time, types A, B, C, D, and E are differentiated. Types A and B were identified by Burke (1919) with the assistance of a reaction neutralizing the toxin with an antitoxin. The botulism bacillus of the C type was discovered by Bengston (1922) in the larva of the fly Lucilla Caesar. A very similar microbe was isolated in Australia by Seddon (1922) from the bones of a cow which had died from bulbar paralysis. He named this microbe Clostridium Parabotulinum. The toxin from the Seddon bacillus can be neutralized by the antitoxin C, although the antitoxin of Clostridium Parabotulinum is not neutralized by the toxin of type C.

Taylor and Robinson (1927) isolated from the skeletons of animals in South Africa a microbe, characterized by antigenic properties of a toxin different from types A, B, and C. The authors called this microbe Clostridium Parabotulinum Bovis. Later, Meier and Cunnison (1929) as well as Weinberg suggested that it be named Clostridium Botulinum D.

In 1937, Kushnir isolated from a red fish in the Sea of Azov a new type of botulism bacillus. The toxin of this microbe could not be neutralized by the antitoxins of the types A, B, C, and D. The author called it Clostridium Botulinum E. During poisoning from botulism, this type of botulism bacillus was isolated by Zavadovskii (1940) from a smoked herring.

1. Spores

All types of the botulism stimulant form spores, which are located on the end of the bacillus and rarely in the middle. The end of the bacillus fans out, and it together with the spore has the appearance of a tennis racket. The spores in the botulism stimulant serve as a protective attachment for the latter under circumstances unfavorable for the stimulant's existence. The stability of spores to the action of various physical and chemical factors is extraordinarily great. Spores can especially well undergo the drying process. According to the data of East and Meier (1922), spores remain alive after 247 days of exposure to a dry condition.

The resistance of spores to heating is dependent upon the composition of the surroundings. In a milieu containing a considerable amount of fat, spores are much more stable to temperature than in a place with little fat. Spores maintain themselves well

when boiled at a temperature of 100 degrees /Centigrade/ over a period of five hours and die some times at this temperature only after six hours. At a temperature of 105 degrees, the spores die not sooner than after two hours; when the temperature is raised to 120 degrees, they die after ten to twenty minutes. These data show that the spores of the botulism bacilli possess a very high degree of stability when boiled. Regardless of the fact that considerable research has been done of the study of the spore's stability under different temperatures, this problem should not be considered as definitively solved. As numerous authors have noted, the spores of the botulus stimulant, under the influence of a high temperature, can pass into a "dozing spores" in cultures and for a very long period of time not germinate at all. The maximum time for such stopped germination was computed by Dixon (1928) as being six months. Burke (1933) observed this stop in the germination of spores, which had been exposed to heat, to last 144 days; the author did not consider this to be the limit. The following relationship between the length of heating time and the germination period of spores was established: the longer spores are heat treated, the slower they will grow. The author considers that superficial sterilization of various materials will not free them in whole from spores of the botulism bacilli, due to the latter's slow rate of growth.

Dixon, Burke, Beck and Johnston (1922, 1925) studied the spores of 11 stems from the botulism bacillus in 37,000 samples over a period of 28 to 29 months. In each sample, there were about 50 million spores with a broth under vaseline oil, with a broth minus the oil, with agar and cerebral surroundings. The spores

were heated to 100, 107, 115, 118 and 121 degrees and then germinated for various periods of time. The maximum period of germination for spores under these conditions was observed to be in a broth under a layer of vaseline and was equal to 37 months. The authors noted that the maintenance of spores under a layer of vaseline increases their resistance to heat and lengthens the periods of germination. This should be necessarily observed during the heating of suspicious canned food containing fats.

East and Meier (1922) consider that young spores, separated from six to ten days from the bacilli of botulism, are more stable in connection with temperature than are spores from older cultures. Dixon (1928) takes issue with this and states that the growth of spores is not affected by their resistance to temperature.

With regard to the development of technology along lines of freezing products, there have appeared lately works devoted to the study of how freezing affects the spores and the toxin of botulism bacilli. Tanner (1936) announced that spores, after having been kept for a long time in a freezing condition, possess the ability to germinate and to create toxin. Such results were also obtained by Streik and James (1935). Weledge and Park (1933) reported that canned goods, contaminated with spores that were free from toxin, became poisonous after freezing. After feeding experimental animals with these canned goods, four percent of the cases became sick with botulism and at times with a lengthy incubation period: from five days to three weeks. In the opinion of the authors, this depended upon the autolysis of the spores during the period of freezing. In their experience, the spores survived well for a whole year a temperature of minus sixteen degrees /Centigrade/.

Segal (1940) in her experiments corroborated the results mentioned above. In the preservation of products, infected with the microbes of botulism, over a period of four months under freezing conditions, the toxin produced by them did not disintegrate. After infecting the products with the spores from botulism, free of toxin, thoy were able to maintain themselves in a frozen condition at temperatures from minus three to minus four degrees. It appeared that in many cases the products (meat, fish, garden strawberries, milk) contained toxin during ten to twenty days of preservation. The author claims that the botulism bacillus did not multiply at this temperature but that a destruction of the spores took place. Consequently, products frozen together with spores can become toxic.

According to the opinion of certain researchers, the type to which spores of the botulism bacillus belong plays an important role in their stability under physical and chemical factors. The spores of type A are considered to be more stable than the spores of type B or C.

The spores of the botulism bacillus are resistant to chemical bacteriocytic substances. So, a ten percent solution of hydrochloric acid kills spores at room temperature after only one hour, a forty percent of formalin in a double dilution kills them after only twenty-four hours. Raising the temperature hastens the functioning of desinfectant substances on the spores.

Slavutskaya (1937) studied the influence of ethyl alcohol upon the spores of the botulism bacillus. In her experiments, the spores maintained themselves alive in the alcohol over a period of two months. Damp spores, introduced into a sturgeon containing 14

percent of table salt, with the maintenance of room temperature, appeared to be alive for two months.

An interesting observation was conducted by Nechaevski (1936) with the planting of spores into the stomach, duodenal, intestibal and pancreatic juice and also in the bile. It appeared that the spores in these juices germinated and in certain instances produced toxin. The solid bile put pressure against the germination of the spores, the diluted bile permitted spores to germinate and to produce toxin.

Regardless of the numerous experiments conducted over the past 25 years with the aim of studying the stability of spores from the botulism bacillus toward the action of various physical and chemical factors, a radical method of destroying them in food products has yet to be discovered. Careful autoclaving of canned goods remains until now the only method for destroying spores. Schoenholz, East and Meier (1923) studied the formation of toxin from the botulism bacillus in different canned stuffs, which they infected with spores. The authors established that the germination of spores in canned foods and the formation of toxin takes place very rapidly.

At the present time, it was definitely concluded that boiling does not destroy spores in products. Burova, Nechaevskaya and others (1935) infected cartilaginous fish with a toxigenic culture from the botulism bacillus containing spores and then boiled it (from thirty minutes to one hour) after which the fish lost its poisonousness: the toxin that had been introduced into the fish from the culture had disintegrated. However, after 36 hours of maintenance at temperatures between plus 15 degrees and plus 17 degrees, the fish again became poisonous. These results explain why it is that

under certain conditions boiled products after a certain period of time precipitate deadly poisoning.

2. Toxin

The stimulant of botulism has the ability to form a very strong toxin in food products, in the organism of a human being and in animals, and under artificial circumstances. The toxin of the botulism bacillus was first obtained in Russia by Anrepa in 1885 fish, which had caused poisoning, and from the organs of human beings who had died from poisoning. In 1896 Van Ermeng obtained toxin from meat.

The toxin from the botulism bacillus has more than three times the power in its action upon the organism of human beings and animals than do other bacterial toxins. It is possible to obtain a botulism toxin that will kill a guinea pig after a dose of 0.0000001 grams. The characteristicx of the toxin from the botulism bacillus which differentiates it from other bacterial poisons appears to be the fact that it does not become digested in the stomach-intestinal tract under the influence of juices which digest food.

As the experiments of schoenholz and Meier (1924) showed as well as those of Bengston (1923), the toxigenic property of this microbe is relatively stable. In cultures the bacilli of botulism can maintain themselves on an equal par with toxigenic and non-toxigenic compounds which determined the way for obtaining the stems from one cell. Sterin (1924) isolated 800 cells. Only 253 of them grew, although all were toxigenic. This shows that not all cultures of the botulism bacillus contain non-toxigenic variants.

Certain authors (Orr, Burke and others) have voiced the hypothesis that under the influence of a high temperature or even spon-

taneously, it is possible for toxigenic microbes of botulism to pass into non-toxigenic variants. Katz (1936), having observed the changed properties of a botulism microbe under the influence of high temperatures, noticed that it lost its toxigenic quality. The research of other authors (Freidson, 1936; Chervyakova, 1940; Slutskaya, 1940), however, showed that the heating process did not condition the change from toxigenic stems into non-toxigenic ones.

Our experiments during the time of heating a sturgeon over a period of 40 minutes to one hour at a temperature of 110 degrees showed that inside of the muscle it was 80 to 90 degrees, whereas no change of cultures from toxigenic to non-toxigenic was observed.

In such a manner, the problem of transforming toxigenic stems into non-toxigenic ones under the influence of high temperatures, which has considerable theoretical and practical importance, should not be considered as sufficiently studied.

According to the report of Doze (1924), the toxic formation in types A and B occurs always providing that the growth of the culture take place in nourishing surroundings. With types C and D, the formation of toxin is under considerable fluctuation (Bernston, 1923; Tayler, 1927).

The toxin from the bacillus of botulism is possessed of considerable thermal stability. This is extremely important to remember when heating various suspicious products. The degree of thermal stability of the toxin depends upon, apparently, the stem which has served in abtaining it and the surroundings in which it is being heated. The research of Van Ermeng (1912) showed that the toxin in European stems of the botulism bacillus disintegrated in the course of a few minutes at 100 degrees, during 30 minutes

at 80 degrees, and in three hours after heating to 53 degrees. Tom, Edmondson and Jilter (1919) observed the disintegration of toxin after 30 minutes at 70 to 73 degrees. In the experiments of Orr, Schoengolz and Meier (1924), the disintegration of the toxin at the same temperatures took place sooner. They note that the toxic quality was lost at 80 degrees after 5 to 6 minutes, at 72 degrees in 12 to 18 dr minutes, and at 65 degrees after 10 to 15 minutes. Chertkova (1938) obtained at 75 degrees complete disintegration of the toxin after five minutes.

Minervin and Silverman (1937), on the basis of the data from L. A. Silver (1932) and his co-workers on the property of certain substances (glucose, sorbite, saccharose, glycerin) to retard the denaturing of albumins at high temperatures, concluded that 43.2 percent to 87 percent solutions of saccharose increase the thermal stability of botulism toxin. Thus, it is possible to state that the thermal stability of toxin is dependent upon its surroundings.

The toxin from botulism possesses considerable stability in relation to the direct sunny world and to air. Under their influence, the toxin does not disintegrate for 118 hours (Schoengolz and Meier, 1924). According to the observations of Moracs (1915), a fluid toxin protected from the world by means of sealed tubes was preserved for 13 years. Kashentseva, Volkova and Komkova (1937) after keeping a fluid toxin for a month in a refrigerator under a layer of vaseline noticed a loss of 50 percent in its activeness, and under a layer of toluene - 75 percent. In the tubes without vaseline, or toluene, the fluid toxin almost completely disintegrated after one month in the refrigerator.

In an acidic surrounding (pH = 3 - 4), the functioning of the botulism bacillus' toxin becomes considerable stringer.

alkalis, on the contrary, weaken this poisonous quality of the toxin: at pH = 7 - 8 it loses up to 90 percent of its toxic properties. The toxin does not dissolve in ether or in chloroform; alcohol weakens its functioning. If the toxin is introduced through the mouth into an animal simultaneously with alcohol, then the animal can be saved from death. Treatment with alcohol of an illness that has already developed (through the mouth or subtaneously) does not provide positive results. In the organism, alcohol weakens the functioning of the toxin only when it is simultaneously introduced with the toxin.

A high concentration of table salt will not dissolve the toxin. An occurence has been described where botulism poisoning took place from a fish containing up to 18 percent of table salt. According to the research of Belouskaya (1939), the formation of toxin is possible durimg a concentration of up to 6 percent NaCl in a product. In the event that the NaCl content is raised from 6 percent to 11 percent, the formation of toxin is sharply curtailed; in higher concentrations, it does not take place at all.

The botulism bacillus, when growing under artificial circumstances and in food products associated with various microbes under certain conditions, forms a very strong toxin. This has given an impetus to the study of the growth and the toxin formation of the botulism stimulant in mixed cultures. It appeared that certain bacteria aided its growth and did not deter it from forming the poison.

In the experiments by Fransilon, conducted in 1925, the staphylococci, streptococci, intestinal and hay bacilli contributed to the growth of the botulism bacillus. Proteins and blue

pus bacilli appeared to be open antagonists of this microbe. On the basis of research by Jordan and Deka (1924, 1926), Bac. Sporogenes and certain stimulants of gas gangrene (Bac. Histolyticus and Perfringens) considerably slow the growth of the botulism microbe and disintegrate its toxin. The influence of lactic acid microbes upon the toxin formation by the botulism bacillus was studied by Glotova and Chebotareva (1938). They noted that the faster and stronger the formation of acid in the presence of B. Casei, B. Bulgaricus, Str. Lactis - the less botulism toxin is formed.

Various authors have received contradicting results in the cultivation of the botulism stimulant from mixed cultures. Thus, for example, Remer (1900), Dixon (1926) write about the favorable influence of the hay bacillus upon the formation of the botulism toxin; Stark, Sherman (1929) report that the hay bacillus is able to destroy the botulism toxin completely. According to the data from Grodko (1940), the presence of cocci, intestinal and hay bacilli, Bac. Sporogenes, proteins, Bulgarian bacilli in a meat-peptic broth and in Tarotstsi surroundings did not impair the toxic formation by the botulism bacillus. The sarcina and hay bacilli in association with the botulism microbe in the same surroundings or in canned corn intensified the multiplication and toxic formation of this microbe.

Meier and Gunnison (1929) showed that the growth of the botulism bacillus and the formation of toxin in sour fruit depend not only upon the biological properties of this microbe but upon the associated micro-organisms which survived the incomplete sterilization.

From the above described experiments, we can see that the influence of various bacteria upon the growth and toxin formation of the botulism bacillus depends not only upon the appearance of and type of bacteria but also upon the composition of the surroundings and the associations of the bacteria which are in the area. Depending upon the combination of these factors, results can differ. That is why with one and the same type of bacteria different authors received varying results.

In connection with the very great strength of the botulism toxin, at the present time research is being conducted in the USA for the purpose of utilizing it for warfare. Rosenbéfry, Sabat and Foldi (1947), in an article devoted to bacteriological warfare and a critical analysis of the means possessed for application in warfare and means for defense from the same, place special emphasis upon the botulism toxin which affects the human organism in fatal doses. The importance of botulism toxin, from the military point of view, is the fact that it can easily be obtained in large quantities, that it distinguishes itself by its high degree of stability and precipitates an immunity during vaccination which provides the possibility for an attacking army to take the necessary precautionary measures. Although up to the present a case of poisoning from water has not been heard of, the authors do not doubt that this is completely possible. They also consider possible the spraying of dry toxin in the form of a powder, the destructive properties of which can cause sickness.

Rims, Cadius, Housewright and Willson (1947) made a detailed study of the immunization of human beings against the botulism toxin. According to the report of these authors, they claim to have obtained

satisfactory results during the immunization of people with fluid and precipitated alum of botulism anatoxin.

On the basis of this research and also the work of Hottl and Abrams (1947), it is apparent that the USA is conducting experiments toward obtaining unrefined and crystalline toxin of botulism.

3. The Distribution in Nature of Botulism Bacilli

For a long time it was thought that the bacillus of botulism was but little distributed in nature. This opinion was first stated by Van Ermeng and remained until 1919 supreme. In the year 1919, Burke published considerable material pertaining to the distribution of the botulism stimulant in nature. It was found by the authoress in fresh fruit, in the soil of orchards, and in fodder. In one case, this microbe was excreted in the feces of a pig. Many of the scientists were interested in the problem: does the botulism bacillus appear in the saprophyte of animal and human intestines or in the stationary dwellers of the soil?

Through numerous experiments it was established that the microbe of botulism is very widely disseminated in nature, especially in the soil. After the study of 634 samples of the soil in California, Meier and Dubovskaya (1921) found botulism bacilli in 30 percent; from 1,638 samples of soil from other states and Canada, the botulism stimulant was present in 24 percent. Other authors, after research on the soil from different states in the USA, have found the presence of botulism bacilli in even higher percentages of the samples. Thus, in the soil of California the presence of this microbe was found in 70 percent of the samples, in the soil of the state of Maryland - in 91.9 percent. The study of the soil for the presence of botulism bacilli was conducted in many countries with

positive results. The latest report by Haines (1942) from England contains data on the study of soil samples from different localities in 16 duchies: from the forest, meadow, orchard, soil. The bacillus of botulism was found in 5 to 14 percent of the samples.

In the opinion of Meier, the virgin soil of forests and meadows, in which type A is met with more frequently, appear to be locations where botulism bacilli are found in nature. Type B is more often found in cultivated, fertilized soil.

We consider it necessary to emphasize here that the statements found in literature to the effect that Bac. Botulinus appears only in stationary dwellers of the soil and only in virgin soil, i.e. in soil where the foot of man or of a warm blooded animal has not stepped, are without any kind of responsible scientific foundation. All of these statements seriously contradict the biological properties of the botulism stimulant (rigid anaerob, a temperature optimum for growth, etc.) as well as all of the epidemology of botulism.

The data obtained from the study of growing products and forrage are characteristic of the soil microflora in relation to the presence of the bacillus of botulism. Meier in his works shows that the spores of the botulism bacillus most often of all are found on beans (34 percent), on decomposing plants (20 percent) and on fermented green fodder (20 percent).

Experiments in the study of the contents of human intestines revealed a quantity of destructuve cases where botulism bacilli were found. Easton and Meier (1924) while studying 88 samples and Kann (1924) with 65 samples of human excrement did not find the microbe in a single instance. Tanner and Deck (1922) while

planting 10 samples of human excrement obtained twice type B of botulism bacillus. In 50 samples of excrement from animals, Easton and Meier found the stimulant of botulism three times in a pig and twice in cattle. Tanner and Deck also came upon the bacillus of botulism in three instances during experiments with pigs.

On the basis of his research, Meier (1928) came to the conclusion that the intestines of a human being and of animals play completely no part in the dissemination of spores from the bacilli of botulism.

However, subsequently he completely repudiated his original argument. In his work (1931), devoted to new data on botulism, he writes that one can consider probable the saprophytic existence of certain different types of botulism bacilli in the excrement of animals. In such an event, the mass of feces from animals may be the cause of contaminating products with the microbes of botulism.

As if in corroboration of this, Henderson (1933) reported the finding of a botulism bacillus in the liver of ducks and others which had not been stricken with botulism. This is proof of the possibility that wild birds can become the carriers of this microbe.

In the view of Jordan and Deck (1924), and also that of Tanner (1922), negative data in the study of excrement for the presence in it of botulism bacilli are often due to the accompanying microflora which strangles the growth and toxic formation of the botulism bacillus. In this connection, Tanner in his work (1940) on food infections and intoxications is in favor of admitting the role of aimals and human beings as carriers of botulism bacilli in the dissemination of botulism.

In the USSR, the bacillus of botulism was found in the soil, in fruit, and also in the intestines of the cartilaginous fish in the Caspian and Azov Seas. These data will be discussed in detail in the chapter on the role of the cartlaginous fish in the dissemination of botulism.

CHAPTER II

EPIDEMIOLOGICAL DATA ON BOTULISM

In the opinion of many authors, botulism appears to be a rare illness. This, however, does not accurately portray reality. In the first place, the cited opinions are contradicted by extensive distribution of the botulism stimulant in nature and the fairly often discovery of it in food products. In the second place, all cases of botulism are not diagnosed at the time and occasionally are not even recognized as such.

The number of botulism cases is usually estimated on the basis of known epidemics. Aside from that, the literature on the subject does not report by far all botulism poisonings. In works devoted to the study of botulism epidemics, the most characteristic cases - either numerically or in their course - are described.

At the present time, it is well known that the cause of botulism in Western Europe, and specifically in Germany and France, is most often due to canned products of animal derivation: ham and sausage products. In the USA botulism epidemics in the majority of cases are precipitated by canned fruit.

In the USSR, botulism poisoning is mostly caused by cartilaginous fish (white grampus, sturgeon).

There is no accurate data on the incidence of botulism in pre-revolutionary Russia or foreign countries. According to the report of Meier, from 1735 to 1924 in Western Europe there were 4,144 illnesses of which 1,271 ended fatally. In England between 1860 and 1926, botulism cases were registered in 75 cases and two ended in death. In the USA, from 1899 to 1926 the number of persons ill with botulism was 1,816 of whom 1,163 died.

According to Meier's figures, in pre-revolutionary Russia and in the USSR from 1818 to 1920 there were 388 cases of botulism of which 183 ended in death. That these figures do not correspond to reality is proven by Table 1, compiled by us on the basis of reports on botulism available in the literature of Russia from 1818 to 1913.

Table 1. The Number of Botulism Cases on the Basis of Data in the Literature of Pre-Revolutionary Russia

Number of Epidemics	Date	Place of Epidemic	Origin of Poisoning	Number of Cases	Number of Dead	Author	Source and Published Date
1.	1818	Yakutsk	Salted Sturgeon	7	7	Edekauer	Fish Poisoning, St.Petersburg,1892.
2.	1826	"	same	7	7	"	Ibid.
3.	1834-1836	Oklaminsk District	"	12	12	"	"
1	1833	Moscow Province	Salted Wh. Grampus	7	7	"	"
3	1836	Moscow District	"	10	10	"	"
2	1836	Ryazan Prov., Pchely Village	"	6	6	"	"
2	1838	Moscow District	"	6	6	"	"
3	1838	Podole Distr., Moscow Province	"	17	17	"	"
2	1838	Saransk and Ryazan Distr.	"	12	12	"	"
7	1838	Smolensk Province	"	51	51	"	"
1	1838	Kaluga	"	3	3	"	"
1	1840	Bronitskii District	"	3	3	"	"
1	1843	Medyn	"	2	2	"	"

1	1843	Lyakhvensk District	"	6	6	"	"
2	1843	Tulskaya Province	"	15	15	"	"
1	1843-1845	Saint Petersburg	" and Sturgeon	18 180	8 -	" Shyuts	" Works of Liberal-Economic Assoc., No. 4, 1845.
1	1846	Astrakhan	Salted Bream	2	2	Berkovski	Military-Medical Journal, XIX, No. 2, 1857.
1	1853		Salted Sturgeon	9	3	"	Ibid.
1	1863	Saint Petersburg (Watch of a Cold Court)	Salted, Par-boiled Wh. Grampus	30	-	Edekauer	Fish Poisoning, St.Petersburg, 1892
1	1863	"	Salted Wh. Grampus	5	5	"	Ibid", 1882.
2	1863	Luga, Nov-aya Ladoga	"	29	29	"	Ibid.
1	1886	Astrakhan	Salted Sturgeon	3	1	Sokolov	Works by Assoc. of Astrakhan Doctors, 1893-1894; pub. 189
1	1887	"	"	1	1	"	Ibid.
5	1888	"	"	9	3	"	"
5	1889	"	"	5	2	"	"
4	1891	"	"	6	3	"	"
6	1892	"	" and Wh. Fish Sturgeon	20	9	"	"
2	1893	"	Salted Sturgeon	2	2	"	"
3	1894	"	"	5	2	"	"
1	1884	"	Wh. Fish Sturgeon	6	1	"	"
1	1885	"	Salted Sturgeon	1	1	"	"

4	1887	"	Salted Wh. Fish Sturg.	11	6	"	"
9	1888	"	Salmon	27	12	"	"
6	1889	"	White Fish	18	5	"	"
2	1890	"	"	3	-	"	"
4	1893	"	"	16	8	"	"
1	1892	"	"	15	-	"	"
1	1893	"	"	2	1	"	"
1	1890	"	Wh. Grampus	11	5	Argstamov	The Physician, No. 19, 1891.
1	1885	Kharkov	"	3	3	Anrep	Ibid., No. 14, 1885.
1	1887	"	Salted Fish	2	2	Livental	Pharmaceutical Journa No. 20, 1887.
1	1883	Rostov on the Don	Herrings	3	2	Chugin	The Physician, No. 2, 1883.
1	1889	Korsun	Salted Sturgeon	1	1	Yakovlev	Messenger of General Hygiene, 1889.
1	1913	Astrakhan	White Fish Sturgeon	2	2	Konstansov	Fish Poison, Petrograd, 1925.
	in all 101			609	283		

For the above period, the Russian press reported 101 epidemics of botulism in the course of which 609 persons were poisoned of whom 283 died (46.4 percent). However, even these data on botulism in Russia appear to be incomplete. In reality, the incidence of illness was much higher. The high mortality date is noticeable prior to the introduction of an anti-botulism serum.

It is necessary to emphasize especially that due to the historic instruction of the Central Committee, All-Union Communist Party (of Bolsheviks), to the Party organizations in the food industry on December 22, 1933 and the directives of the Soviet government - the sanitary condition of

enterprises in the food industry was rapidly improved. This led to a considerable drop in the cases of botulism and in relationship to certain products (canned goods) to its complete liquidation.

As a result of the wide distribution of refrigeration in the fish industry and in fish combinats, the preservation of cartilaginous fish has been largely attained in frozen condition after which it is transmitted through the commercial network in a fresh state. Such a fish, undergoing immediate cooking, as has been shown in practice is not the cause of botulism.

In such a fashion, the development of refrigeration industries in our country has become an important factor in the rapid lowering of incidences of botulism.

1. The Planting Quality of Products from Botulism Bacillus

As we have already seen from the data in the literature on the subject, the stimulant of botulism is considerably distributed throughout nature. For this reason, all agricultural products, dirtied by the soil, may contain the spores of this microbe. The quantity of spores also depends upon the sanitary conditions and the technological processing during canning.

Through the work of a number of authors in the USA broad infiltration has been established in various fruits under real conditions and also in canned fruit the bacilli of botulism. In our own country, the literature on the subject contains notes on the infiltration of fish, fruit and preserves by the spores of the botulism stimulant.

Burovoi, Nasledyshevoi, Nechaevskaya, Kats and Lemisovaya (1935) were able to conduct bacteriological observation of different products: meat, fish, fruit, and canned fruit and semi-preserves. All told there were 529 samples, of which 307 were canned samples and 192 were samples of salted and frozen cartileginous fish. The bacilli of botulism were

discovered in 17.5 percent of the fish, in 8.3 percent of fish in tin cans, and in 2.7 percent of the fruit samples. In 28 cans of meat, the microbe was found four times. Biological and bacteriological analysis on 44 samples of fish roe, taken from 16 liter-size bottles which had been partially freed from the product, showed that four of the bottles contained the microbe of botulism (Brun, Lorber, and Burnos, 1937). The fish roe from certain of the bottles was the cause of botulism epidemics.

Zayats (1936) checked for the presence of this microbe 215 specimens of fruit and 25 samples of the soil in the orchards of Dnepropetrovsk. The fruit was taken fresh for bacteriological analysis and compared after six months of preservation under various conditions. Six toxigenic strains of the botulism bacillus were determined: three from potatoe, one from radish, and two from the orchard's soil.

In Rostov-on-the-Don, Vatolkina and Itonieva (1938) conducted an analysis of fish, auxiliary materials, and plants with edible roots for the presence of the stimulant of botulism. In 340 samples of fish and other ingredients, 60 strains of rigid anaerobes were found. All of these, in their morphological and cultural characteristics, appeared very similar to the bacillus of botulism. During the study of 333 specimens of plants with edible roots (carrots, parsnip and parsley), 15 strains were obtained which provided an agglutination reaction with serum against the bacilli of botulism types A and B. Certain of these strains possessed toxigenic qualities.

The research of Moreinis (1942) provides proof that the bacillus of botulism [illegible] in the infection of fish and other products is helped in this process not only by the conditions of sanitary service but also by the house-keeping activities of man (dirtying the water mains with discarded items, feces of animals, etc.). The author examined

the entrails of 239 fish from the Barents Sea (salmon, cod, haddock, herring) but did not find one stimulant of botulism in them. However, he did discover a toxigenic strain of this microbe in one (out of 16 samples) pike perch from the Sea of Azov.

The report by Gimmelfarb, Bershtein and Gordian (1941) on the results of a bacteriological analysis of canned goods shows the important part played by various anaerobes, among which are also the bacilli of botulism, in the contaminations of products. According to their data, in the course of the years 1935-1937 the sectional laboratory of the Ukrainian canning trust checked 53,112 cans for sterilness. On the average, 12 to 14 percent of these were found to be unsterile. Decay inducing anaerobes were present in 0.2 to 0.3 percent, i.e. a total of 250 cultures. Forty-two cultures were placed under careful analysis. Certain of these underwent agglutination with the serum of the botulism bacillus. The authors consider that this was a group reaction of agglutination.

As has already been mentioned, the reaction of the surroundings and the accompanying microflora have considerable influence upon the development of the botulism bacillus in products. These factors can restrain or facilitate the multiplication of the microbe. Apart from this, the concentration of table salt in the product and the temperature at which the latter can be preserved are both of importance.

Interesting results were achieved by Zaslavski and Chervyakovaya (1940) in their study of the conditions necessary for the formation of toxin in canned tomato juice and various fruit juices. After the contamination of these juices by the spores of the botulism microbe over a period of 12 months under conditions where the products were kept at room temperature and at 37 degrees /Centigrade/, the formation of toxin was not observed. However, in the event that efflorescence developed

in the products, the formation of toxin took place, the presence of which was established by means of the neutralization reaction.

During the past few years, the formation of toxin from the botulism bacillus has been established in frozen products, providing that they contained spores of this microbe when being frozen. Experiments described in literature, in the course of which meat, fish, canned fruit are contaminated with spores and then frozen showed that after defrosting these products became poisonous in connection with the destruction of certain parts of the spores. The presence of the toxin in frozen products was established in the works of Voivod (1939) abd Segal (1940) after biological tests.

Fairly often, the products contaminated with the stimulant of botulism and containing toxin do not inspire the opinion of good quality on the basis of their ex... al appearance. This cardinal fact was brought out by Burova, Glotova, Minervin and others. In the study of botulism epidemics, this was observed many times. It should be mentioned here that botulism epidemics are caused in their majority by lower quality products, especially various fish.

At the present time, bacteriological analysis is applied for the identification of botulism bacilli in food products. The presence of toxin is determined by means of a biological test, during which it is imperative to apply the neutralization reaction with anti-botulism serum.

In order to rapidly identify botulism toxin in canned and other food products, Nasledysheva and Braslavskaya (1935) suggested the precipitation reaction. This reaction can be set up with an extract from the products and serum prepared on rabbits, immunized by an anatoxin and the culture from the botulism bacillus. In checking on this reaction, Kantsur and Chertkova (1940) came to the conclusion that it is a specific

one and that in its sensitiveness is inferior to the biological test. The research of Domatchenko (1940) produced contradictory results. The precipitation reaction appeared positive with filtrates of canned goods that had been contaminated by Bac. Botulinus. This was also the case with filtrates of canned goods contaminated by the bacillus of sporogenesis, through which its non-specificity was established.

In connection with the fact that a bacteriological analysis of products requires considerable time, Voivod and Kremer (1940) suggested that in order to discover the bacillus of botulism, mixed cultures should be submitted to the agglutination reaction. On the basis of the data obtained by these two, the analysis time for products under these circumstances is shortened to between two and three days.

Thus, the problem of quickly determining the presence of toxin and the bacillus of botulism in various products remains unsolved and requires further study.

2. The Role of Cartilaginous Fish in Disseminating Botulism

In pre-revolutionary Russia poisoning from fish was known in the far past. During certain years, this illness was distributed widely. For a long time, the cause of the poisoning was unknown. In his monograph Fish Poison, Edekauer (1882) reports on poisoning from fish in the years from 1818 to 1863. For this period, he mentions only four cases of poisoning from raw salted sturgeon. The remaining poisonings about which the author writes were precipitated by white grampus in salted condition. Fish poisoning occured in 33 populated points in Russia; 205 persons died; the number affected is reported by the author only in fragmentary cases. Thus, in 1843 in St. Petersburg at an educational institution 18 persons were poisoned from salted white grampus; 8 of these died. In 1863 some 30 persons in the watch of the cold court

were poisoned from salted paracooked white grampus, the number of deaths being unavailable to the author. There were also cases of poisoning from fish in Moscow and in other heavily inhabited points of the country.

In his work, Edekauer emphasizes that the number of cases of poisoning from fish was considerably higher than the official data which he which he cites. The description of the diagnosis for these poisonings is very similar to the diagnosis of botulism. As a method in the struggle against fish poisoning, he recommends the cooking of suspicious salted fish. A second author - Berkovskii - in an article "About Fish Poison" (1857) also describes the poisoning of nine persons at Astrakhan from sturgeon; three of these died. Apart from that, he mentions the fact that German and French periodicals also carried reports on poisoning from fish during this time. The physicians Teleb and Demarti in the Revue Therapeutique de Médicine (1852) wrote on the death of several persons who had been poisoned by flounder and about the deaths of six people poisoned from marinated fish on a British man-of-war. The physician Shevale in the Revue Thérapeutique Médico Chirurgique (1856) describes the death of 34 persons out of 42 who had eaten salted fish on a whale boat. Consequently, poisoning from fish during this period was not only true in Russia. Berkovskii in his article provides a detailed description of the clinical card for the poisoning which is very similar to the symptoms of botulism. In order to emphasize this, I am quoting an extract from his article:

"The first cases of illness made their appearance not earlier than one hour and not later than five hours after the meal. This time did not depend upon the condition, growth or field but only upon the quantity of the poison. No matter what the number of persons who participated in the meal, they all became ill at more or less the same time. The seriousness

of the illness is determined by the quality of the poison, which does not change as does the quantity. Thus, in one instance a 17-year old youth, a 45-year old athletically consituted man, a 70-year old sick woman with swollen legs and water in her stomach, and several middle-aged women all became ill simultaneously after five hours. The intensity of the poison is extraordinary, if taken into consideration that the quantity of fish from which people died was very limited, since a fish that has been salted a great deal and much could not be eaten. Therefore, it could not have weighed over one pound. The fat in which the poison is contained does not weigh more than two "zolotniks" (8.532 grams in all). At first the feeling is one of unexpected unpleasantness, stomach cramps, then spinning of the head, and a blearing of the eye-sight. Following these comes a sharp tearing pain in the stomach and a cutting in the breast and throat. The pain in the stomach after a short period of time ceases, but it comes back with increased power. The ill person throws himself from side to side, lies on his stomach, squeezes the stomach walls against the spinal column toward which they themselves are gravitating, does not desire to vomit but suffers a pain inthe small of the back and below the right intestine. Besides a burning sensation, the patient believes that weights are on his chest. He is in no condition to take a depp breath, since breathing becomes more and more difficult with time. Anxiety increases. The voise, at first strong, slowly fades away. The pulse in the beginning of the illness changes but little. The patient is plagued by thirst, but he is unable to swallow freely from the start, especially cold drinks. Hot beverages are swallowed with eagerness, but soon that is also impossible, because each drop of liquid causes a severe shortness of breath and convulsions in the throat. The eyesight becomes extinguished, the pupils are dilated, the eyelids undergo paralysis and

can be lifted only with the fingers. The higher extremities are weakened, while the lower can not be lifted at all. The compartments are shortened. Breathing becomes more difficult and short, especially inhalation which towards the end gradually stops. The movement of the chest can no longer be noticed. Before death, all pain disappears. The patient lies motionless, silent. The heart beat is weak, loss apparent and transfers into palpitation which continues for several seconds although breathing has completely ended.

During all of this time, consciousness remains clear and is preserved until the last minute."

It is interesting to note that currently during botulism poisoning these same symptoms are described and that doctors emphasize the preservation of consciousness until the very last minute by those dying from botulism.

Berkovskii shows that a cooked fish will not cause poisoning. In this connection, the Russian army at this time was issued a special instruction forbidding the requisition for food of salted sturgeon, white grampus or sturgeon that was fresh.

In Astrakhan, the sanitation doctor Sokolov (1896) occupied himself with the collection of materials on fish poisoning. He reports about 150 cases of poisoning from fish in this city from 1886 to 1894. Of these, 57 died. The majority of cases were caused by fresh salted sturgeon, whereas only a few came from fresh salted white grampus. Symptoms of the poisoning usually appears after 10 to 12 hours, in rare instances later, and in certain cases after only two hours from the time food wa taken. Sokolov provides a detailed description of each case. Two abbreviated examples are given below in order to compare them with present clinical treatment.

1. 1886. Bulychevskii, 32, poisoned by raw salted sturgeon. Illness began after 18 to 20 hours. Nausea, vomiting, spinning of head, loss of sight, dilation of pupils, ptosis, dry tongue and pharynx, constipation, stoppage of urine, general muscular weakness, anxiety, tightening in chest, pulse 90-96, shortness of breath, paleness of face and integument, consciousness clear, temperature normal. Dies after 2½ days.

2. 1894. Malitskii Mikhail, 38, poisoned from raw salted sturgeon. Beginning of illness 12 to 14 hours after food was eaten. Nausea, vomiting, dryness of tongue and pharynx, normal temperature, pulse 80-86-90, somewhat faster breathing, constipation, impediment to urination, dilation of pupils, foggy sight, softening and ulceration of both corneas, difficult swallowing, coughing attends swallowing, loss of voice, consciousness clear, insomnia, strong general weakness, anxious condition.

From the above cited descriptions, it is clear that they are very similar to a current description of botulism. The clinical diagnosis of all other poisoning about which Sokolov writes is the same as those quoted. In analyzing all cases of poisoning from the cartilaginous fish in Astrakhan from 1886 to 1894, it is apparent that of the 13 children below 16 years of age, 11 died (85 percent); of the 137 adults, 46 died (33 percent). Therefore, children under 16 years of age proved to be three times as susceptible to fish toxin as were the adults.

In the eighties of the last century, the study was begun of causes for the poisoning of the human being by cartilaginous fish. Numerous experiments were conducted on this problem by Anrep (1885) and Livental (1886). On the basis of chemical analysis of the

cartilaginous fish, they came to the conclusion that the poisonous substance in the fish were the ptomaines. Anrep was especially active in the study of the causes for poisoning from fish. In 1885 he obtained by using the Stas method some kind of a poisonous substance from a fish which had poisoned several persons (three of whom died). The author called it ptomaine. This substance was proven to be very poisonous for dogs, frogs and rabbits and resulted in their ppisoning which was similar to the poisoning of human beings from cartilaginous fish. Such a poisonous substance was discovered by Anrep in the stomach content and intestines, in the liver, blood, brain, and spleen of a man who had died from poisoning by a fish. On the basis ofhis experiments, the author comes to the following conclusion:

"Obtaining identical ptomaines from so many substances differing in their composition, like fish, the organs and urine of those poisoned; also, to which I attach great significance, requiring so many different methods for obtaining them, like the Stas' and Briegr" methods; further, convincing myself of the substantial invariability of my ptomaine and in the similarity of phenomena after poisoning from fish in people with the phenomena in animals after they had been poisoned with ptomaine; I could only come to one conclusion - acknowledge that fish toxin was identical with the ptomaines found by me."

The poison that was discovered by Anrep in fish and in the organs of those who had died from fish poisoning, would dissolve on boiling and under the influence of alkali. The experiments of Anrep were corroborated by Livental. On the basis of these data, which the authors cite as characterizing the substances discovered by them, it is possible to conclude that this was not ptomaine but the toxin of

botulism. This is substantiated by the similarity in symptoms for the poisoning animals and human beings from cartilaginous fish, which in turn is very reminiscent of the diagnosis of botulism. Apart from that, the poison obtained by Anrep dissolved when heated and under the influence of Alkali, which was also very characteristic for the toxin of botulism. As we have already mentioned, Anrep was the first to isolate the toxin of the botulism stimulant in pure form even prior to the time when it was obtained by Van Ermeng.

In 1891, Argustamov attempted to explain the bacterial nature of poisoning by cartilaginous fish. For this purpose, he studied 11 cases of poisoning from fish, of which five were fatal. From the cadavers of the dead, he obtained a pure culture of some kind of microbe. He isolated this microbe from the fish which was the cause of the poisoning. Separations from the microbe killed rabbits but did not kill dogs and cats, which would become sick and recover. The old cultures killed rabbits faster. Cultures, submitted to lengthy boiling, also killed animals. This proved that the isolated microbe had nothing in common with the botulism microbe, the toxins of which as is known very quickly dissolve after boiling.

Chugin (1883) describes that poisoning of three children from herrings, the diagnosis of which was very similar to botulism (two of the children died).

Popov (1887) also worked on the causes of poisoning from fish.

Ziber-Shumova (1894), Zabolotnyi (1914) and other authors were unsuccessful in their study of the bacterial nature of fish poisoning.

Interesting work was conducted by Konstansov (1915). The author isolated from fish, which had caused the poisoning of people, a microbe which morphologically was very similar to the bacillus of

botulism, discovered by Van Ermengy, from which the former differentiated itself by certain cultural and biological characteristics. It was apparent that both microbes belonged to one and the same type. This microbe was named Bac. Ichtiismi by Konstansov. Its toxin functioned very strongly and overwhelmingly affected the nervous system of animals, causing an illness very much like botulism. During the immunization of horses, Konstansov obtained an antotoxin which neutralized the toxin from Bac. Ichthiismi. A crossed reaction of neutralization with the toxin of the botulism bacillus was not established. After the experiments of Konstansov, the problem of the stimulant for poisoning by fish did not obtain a final solution. It became clear that the cause of fish poisoning appears to be a microbe, possibly similar to the stimulant of botulism and perhaps even identical with it. Ruchkovskii in his research (1928) notes the prevalence of sturgeon in connection with poisoning from fish. Studying the functioning of toxin from the botulism bacillus upon various species of fish, he explained that sturgeons themselves are completely unaffected by the toxin from botulism. The author stated the hypothesis that the etiological beginning of fish poisoning appear to be microbes of the Bac. Botulinus type.

A final explanation of the causes for poisoning from fish was made in 1935, through the experiments of Soviet authors - Burovoi and his co-workers and Glotoboi and his collaborators. They proved that fish poisoning is of a botulism nature. These researchers established that the reason for fish poisoning are the type A or B bacilli of botulism.

In 1935, Burova and Nasledysheva published a work in which they announced the isolation of seven strains of the botulism microbe from

the intestines of a cartilaginous fish. These strains were non toxigenic and were identified by the agglutination reaction. In the same year, Glotova, Kibalchich, Komkova and Muromtsev analyzed 166 specimens of cartilaginous fish from the Caspian Sea. They discovered in the intestines of four fish a toxigenic microbe of the botulism type. Apart from this, they also isolated the bacillus of botulism from the intestines of 10 from among 67 specimens of dead cartilaginous fish. Certain of these strains were also toxigenic.

Burova, Nechaevskaya, Kats and Denisova (1935) in their research devoted to the study of the reasons for botulism from fish in the Sea of Azov, specifically emphasize the considerable susceptibility of cartilaginous fish to the microbes of botulism. The authors studied the dissemination of the botulism microbe along the coast of the Azov Sea and discovered it in 150 soil samples (2 percent), in 69 samples of sea water (2.8 percent), and in 49 samples of sea silt (2.04 percent). It is interesting that there is no great difference in the examination of the soil, water, and silt. The microbe is found in the same quantity always.

Simultaneously, the data from the Ukrainian Institute named after Mechnikov (Burova and Nechaevskaya) and the Moscow Institute named after Erisman (Glotova and Soloveva) showed that the contamination of cartilaginous fish with the botulism microbe can go up to 18 - 20 percent.

The discovery of the considerable degree of contamination of cartilaginous fish inspired certain researchers to study the conditions under which the toxin is formed in fish. Burova and Nechaevskaya (1935) established the possibility of toxin formation by the bacillus of botulism in cartilaginous fish under conditions of its contamination

by the spores of that microbe. They corroborated the long known fact that a fish cut into pieces 3 to 4 centimeters long and cooked for one hour at 100 degrees becomes non-toxic. The poison in the fish after boiling becomes dissolved, but the spores remain alive. Of considerable interest are the data on the appearance of toxin in the same fish again after one and a half days at a temperature of 15 - 17 degrees. This should always be remembered when utilizing fish as food, when it is infected with the spores of botulism bacilli. The total harmlessness of the spores from this microbe in their affect upon the human being has yet to be proven.

Komkova also studied the conditions of toxin formation by the bacilli of botulism in cartilaginous fish. After contaminating a fresh cartilaginous fish with spores not having any toxin, she discovered toxin in the fish' muscles one day later at a temperature of 37 degrees and two days later at a temperature of 17 - 19 degrees. In heavily salted sturgeon, toxin is not formed after contaminating them with spores. For this reason, the author comes to the hypothesis that toxin is formed in a fish after the catch and during the time it is preserved and segregated for salting.

Kushnir, Lorbor and Paikina (1937) studied this problem through experiments and came to another conclusion. They consider that the "production of the toxin, in contrast to the opinion of certain authors, cannot precede the salting process but may under certain conditions transpire during the first days after the process when the concentration of salt in the muscular mass is still insufficiently high to block the growth of spores and the formation of toxin."

Under natural conditions, we think that the formation of toxin takes place in the larger part prior to the salting of the fish. However, it can also be created after the salting process, and this

depends upon the temperature of the surroundings and the concentration of the salt. When the temperature is low and the concentration of salt high, toxin will not form after the salting process.

A large amount of research was devoted to the study of the causes for the contamination of cartaliginous fish by the bacillus of botulism under ordinary conditions. In studying this question Burova, Vats and Denisova (1935) came to the conclusion that the infection of the cartilaginous fish with the microbe of botulism takes place exclusively by the endogenic path, from the intestines. The authors attempted to support their argument through numerous experiments. They took samples the cartilaginous fish immediately after it was brought from the sea to the dock. Two samples were obtained from each fish: one from the corner muscles, the integumentary, and the areas of wounding; the other from the internal muscles adjacent to the intestines of the fish. Altogether 82 samples were selected from 82 fish. In five of the samples (12 percent), taken from the muscles near the intestines, the botulism microbe was discovered; it was not found in the samples from the corner integuments.

Nasledysheva and Burova studied the ways by which the bacillus of botulism penetrates from the intestines into the muscles of a fish, under experimental conditions. The tests were conducted upon 32 sturgeon, infected with the spores of that microbe through the mouth. It was established experimentally that the bacillus of botulism penetrated through the wall of the intestines in the fish on the fifth day it was in sea water. This corroborates the fact long known to fishermen that one can become poisoned only from a fish which has remained on a hook in sea water for a long time after death. Burova and her collaborators looked upon the intestines as the main source of infection for the fish, and them recommended that they be removed from the fish sooner.

Kibalchich, Komkova and Muromtsev (1935) studied the conditions contributing to the infection of cartilaginous fish by the microbes of botulism and came to a different conclusion. Not excluding the possibility of contamination by the stimulant of botulism from its intestines, the authors consider "that the gates of entry for the penetration of Bac. Botulinus into the fish tissue are both the endogenic factor (from the intestines) as well as the exogenic (from the corner wounds)." In their opinion, the infection of the fish takes place both ways during incorrect catching and non-sanitary conditions during the preparation of the fish.

Over a period of three years (1936-38), we also conducted a study of the causes for the infection by botulism microbes of the cartilaginous fish. The results of this observation are given below.

Other research on botulism was devoted to the question of how and what role the fish plays in the dissemination of this disease. It is known that in certain instances, the cause for botulism came from a fish: herring, white grampus, bream, salmon (only those types of fish are cited, poisoning from which has been described in literature).

In order to corroborate the possibility of a fish being infected by the bacillus of botulism, Tikhomirov (1935) conducted a bacteriological analysis in the course of which he was able to isolate eight strains of the stimulant of botulism. Four of these were found to be toxigenic, whereas four were non-toxigenic. Muromtsev (1935) and Poslykovskii precipitated an experimental illness in a fish through spores of the botulism microbes. The tests were conducted on specular carp, to which the spores were introduced through the mouth. It was established that the spores introduced into the fish preserve their capacity to live in a dead fish. Spores were found in the muscles of a carp 12 hours after it had been infected.

These experiments were corroborated by Kushnir, Lorber and Paikinaya (1937) who, besides cartilaginous fish, also made tests on the goby, carp and the 'ram.' The authors, in our opinion, came to a completely correct conclusion. They conclude that the infection of sturgeon by microbes of botulism is not connected with the biological characteristics of this type of fish. Apparently when catching fish on hooks, they are inflicted many wounds which appear to be the gates of entry for the contamination of the fish with various microflora having also the microbes of botulism. The conditions of the catch, preparation, transportation and preservation apparently have considerable influence for the infection of fish with the microbes of botulism.

3. Causes for Infection of Cartilaginous Fish with Botulism

In the Soviet Union, the principal cause of botulism epidemics seems to be the cartilaginous fish - Acipenseridae (sturgeon - Acipenser güldenstädti, white sturgeon - Huso-huso, another kind of sturgeon - Acipenser stellatus), which under normal conditions may contain the stimulant of this disease. For this reason, the study of the causes for the infection of cartilaginous fish with the microbe of botulism has for a long time attracted the attention of Soviet microbiologists.

In current research, the material is cited which was collected during the period of bacteriological control of the cartilaginous fish for its infection with the stimulant of botulism. The work was conducted in 1936-1938 on one of the refrigerators and also in the fish combinat at Moscow. In 1936, 470 tests were undertaken on samples of sturgeon of which 11 cases (2.3 percent) contained the stimulant of botulism (table 2).

Table 2. Occurence of the Botulism Stimulant in Cartilaginous Fish, Obtained on the Basis of Tare in 1936

No.	Type of Fish	Number Samples Used	Botulism Stimulant Found	%	Toxin Found in	%	Comments
1.	Fresh-frozen Sturgeon	34	0	0	0	0	Each sample was taken from one specimen
2.	Salted Sturgeon	138	6	4.3	3	2.1	Third grade
3.	Fresh-frozen White Sturgeon	64	1	1.5	0	0	Each sample was taken from one specimen
4.	Salted Wh. Sturgeon	148	4	2.7	4	2.7	Third grade
5.	Fresh-frozen 'Sevryga' Sturgeon	56	0	0	0	0	Each sample was taken from one specimen
6.	Salted 'Sevruga'	30	0	0	0	0	
	Totals	470	11	2.3	7	1.5	

The toxin was contained in three specimens of the sturgeon and in three samples of the white sturgeon, both types having been salted industrially. This fish was of third grade quality, preserved in the raw with a salt content of 14 to 18 degrees. Under such a salt concentration, the formation of toxin does not take place. Apparently it was formed in the fish prior to salting, in the factory. The other fish was of good and satisfactory quality. It was obtained on the basis of tare - frozen in bast sacks and crates, the salted in kegs. Of the 154 samples of frozen cartilaginous fish, the stimulant of botulism was isolated only once.

During the years 1937-1938, a total of 788 analyses were conducted on specimens of sturgeon and white sturgeon. The samples of 367 were taken from fresh or salted fish, obtained on the basis of tare. In these, the stimulant of botulism was isolated seven times (1.9 percent) in the

three types used. Toxin was discovered in one type of third grade quality that had been salted industrially (table 3).

Tbale 3. Occurence of Botulism Stimulant in Cartilaginous Fish, Obtained in Tare during 1937-1938

No.	Type of Fish	Number of Samples	Botulism Stimulant Found	%	Toxin Found in	%	Comments
1.	Fresh-frozen Sturgeon	61	0	0	0	0	
2.	Salted Sturgeon	120	4	3.3	4	3.3	Third grade
3.	Fresh-frozen White Sturgeon	79	2	2.5	0	0	Each sample taken from one specimen
4.	Salted Wh. Sturgeon	50	1	0.5	0	0	
5.	Salted 'Sevruga'	57	0	0	0	0	
	Totals	367	7	1.9	4	1	

Of the 421 specimens, taken from separate samples of freshly frozen fish which had arrived on the basis of tare, the microbes of botulism were isolated 59 times (14 percent). In many examples from this batch the external integuments had been damaged and soiled with dirt (table 4).

The cited data clearly shows the greater (almost seven times) contamination of cartilaginous fish transported and preserved without tare. It follows that the infection of cartilaginous fish with the microbe of botulism depends upon sanitary conditions in the catch, processing, transportation, and preservation of the product. If a fish which contains the stimulant of botulism in its intestines does not have these intestines removed soon after it dies, then as Burova and her associates correctly point out, the microbe will penetrate into the

muscles. Even if the intestines were quickly removed but the fish was transported and preserved under bad sanitary conditions, then it may also be strongly contaminated with the microbes of botulism. Thus, the infection of fish can take place endogenously as well as exogenously.

During the process of bacteriological control, we established that cartilaginous fish of various types was contaminated to a large degree by the stimulant of botulism. This should necessarily be taken into consideration when utilizing the product as food.

Table 4. Occurence of the Botulism Stimulant in Cartilaginous Fish, on the Basis of Tare (in Bulk) for 1937-1938

	Type of Fish	Number Samples Used	Botulism Stimulant Found	%	Toxin Found in	%	Comments
1.	Fresh-frozen 'Osetr' Sturgeon	110	12	10.9	0	0	Each sample taken from one specimen
2.	Fresh-frozen 'Beluga' Sturgeon	174	30	17.2	0	0	ibid.
3.	Fresh-frozen 'Sevryuga' Sturgeon	137	17	11.6	0	0	ibid.
	Totals	421	59	14.0	0	0	

It seems to us that in the utilization in food of products contaminated by the spores from the botulism bacillus, it is necessary also after cooking them to maintain a great degree of care.

Burova reports (1939) that eight workers in her laboratory over a period of 3½ months used cartilaginous fish in food, containing the spores of botulism stimulant, without any adverse effects upon their health. However, even if these persons did not become

openly ill, this does not signify that the spores were completely harmless to their health. As is known, they can penetrate through the walls of the intestines into the internal organs. In this connection, it should be mentioned that the distribution of botulism stimulant in cartilaginous fish of different categories (batches) can be exceptionally varied (see tables 5 and 6).

From the preceding, it follows that products strongly contaminated with the botulism stimulant should undergo special processing, which would guarantee the destruction of spores, prior to their utilization in food.

During our work in the refrigerator and in the fish combinat, some 1,258 specimens of cartilaginous fish were examined. The stimulant of botulism was discovered in 77 cases (6 percent). In the 1,258 expermients conducted on 2,516 mice, for the purpose of finding toxin in the muscles of fish, only 11 of these tests (0.8 percent) were positive. The presence of toxin was established only in third grade fish that had been industrially salted.

The distribution of the botulism microbe in cartilaginous fish was established as follows. Of the fish specimens weighing from 300 to 500 grammes, taken as a basis, the samples were prepared in two retorts with Tarotstsi broth (150 to 200 cubic centimeters each) under a layer of vaseline. One of the retorts was heated at 80 degrees for 20 minutes, whereas the other one was not subject to the heat treatment. The two containers were maintained at 37 degrees for 7 to 8 days, after which the morphology of the formed microbes was checked as was their toxigenousness. A pure culture was then isolated by means of an agar column. Ascertaining that the isolated strains belonged to the stimulant of botulism was conducted by means

Table 5. Occurence of the Botulism Stimulant

in Cartilaginous Fish, on the Basis of Tare in 1936-1938

No.	Type of Fish	Number of Samples Used	Botulism Stimulant Found in	%	Comments
1.	Salted 'Osetr' Sturgeon	15	6	40	All specimens of third grade quality
2.	Fresh-frozen 'Beluga' Sturgeon	20	1	5	Each sample was ta' from one specimen
3.	Salted 'Beluga' Sturgeon	13	4	30.7	All specimens of third grade quality
4.	Salted 'Osetr' Sturgeon	15	4	26.6	ibid.
5.	Fresh-frozen 'Beluga' Sturgeon	10	2	20	Each sample was taken from one specimen
6.	Salted 'Beluga' Sturgeon	10	1	10	
	Totals	83	18	21.1	

of studying their fermentation properties in gellatine and broth with pieces of chicken albumin. Apart from this, reactions toward neutralizing the toxin and agglutination were applied. Of the 77 isolated strains, some 53 appeared to be toxigenous and 24 non-toxic. The toxin in all 53 strains could be neutralized well by a polyvalent anti-botulism serum (A + B), obtained from the anaerobic section of the Central Institute for Epidemology and Morphology (TsIEM) at Moscow. Determination of the types to which the strains belonged was ascertained by the agglutination reaction with serums of the A and B types. The agglutination reaction was simultaneously conducted with the serum of Bac. Sporogenes and Bac. Putrificus. A positive reaction to agglutination was registered by 63 strains with the

the serum of type A and 14 strains with the type B serum. Apart from that, 11 strains were isolated which were similar to the bacillus of botulism in their morphological and biological properties. The toxin of these strains killed mice and small pigs in little doses, but it was not neutralized by the polyvalent (A + B) serum, and the agglutination reaction with the typical serums A and B was negative.

Table 6. Occurence of Botulism Stimulant in Cartilaginous Fish, of Different Types, Obtained without Tare (in Bulk) in 1936-1938

No.	Type of Fish	Number of Samples Used	Botulism Stimulant Found in	%	Comments
1.	Fresh-frozen 'Osetr' Sturgeon	15	3	20	Each sample was taken from one specimen
2.	ibid.	10*	7	70.0	ibid.
3.	ibid.	10	1	10.0	ibid.
4.	ibid.	10	1	10.0	ibid.
5.	Fresh-frozen 'Beluga' Sturgeon	15*	8	53.8	ibid.
6.	ibid.	10*	7	70.0	ibid.
7.	ibid.	10	3	30.0	ibid.
8.	ibid.	10	3	30.0	ibid.
9.	ibid.	13	9	69.0	ibid.
10.	Fresh-frozen 'Sevryuga' Sturgeon	5	2	40.0	ibid.
11.	ibid.	7*	6	85.7	ibid.
12.	ibid.	10	1	10.0	ibid.
13.	ibid.	10	2	20.0	ibid.
14.	ibid.	10*	6	60.0	ibid.
	Totals	145	59	40.7	

*Samples taken from a third grade quality fish.

In the course of the bacteriological control over the 'sevryuga' sturgeon, it was subjected to hot smoking. The influence of high temperature upon the botulism stimulant inside the fish was studied in this connection. According to the report by Kats (1936), a high temperature may serve as a means for transforming toxigenous strains into non-toxic ones.

Prior to the smoking process, a piece of the 'sevryuga' sturgeon was cut off for bacteriological analysis. After this the fish was subjected to hot smoking, during which it was kept in a chamber from 40 minutes to one hour at 100 - 110 degrees. A sample was again taken from the smoked fish for bacteriological analysis. In cases where the stimulant of botulism was discovered before curing and afterwards, a comparison of the toxigenous properties of both strains was made. Prior to the hot curing process, the botulism microbe was isolated from seven 'sevryuga' sturgeons, and all possessed toxigenous qualities. After the smoking, a non-toxic strain was found in one strain; in six of the 'sevryuga' sturgeon, the isolated botulism microbe was not distinguished in its toxigenousness from the strains isolated after the application of the high temperature. Thus, a loss of toxigenousness by the botulism stimulant, under the influence of high temperature in the smoking process, could not be established by us.

In summing up the data on the bacteriological control over cartilaginous fish for the microbe of botulism, over a period of three years, it can be considered as established that the contamination of the cartilaginous fish by the botulism microbe may originate not only endogenously - from the intestines, as claimed by Burova and her collaborators - but also exogenously, from the external surroundings. The endogenous way of infection

apparently does not have much significance. Of decisive importance in the contamination of cartilaginous fish with the stimulant of botulism are the poor sanitary conditions in processing, transportation and preservation.

In the dissemination of the stimulant in cartilaginous fish, the basic role is played by the Bac. Botulinus A which is spread out more in the USSR than types B or E. This should necessarily be taken into consideration during the therapy of botulism with serum.

When purchasing cartilaginous fish in food enterprises, it should always be remembered that the occurence of the botulism stimulant in the various brands of cartilaginous fish may be very high. For this reason, a fish which is strongly contaminated with spores should be subjected to processing that would guarantee their destruction prior to utilization as food or else discarded as unfit to eat. In the utilization of such a product even after cooking, the possibility of infecting people with the bacilli of botulism should not be discounted. How great is the danger from this phenomenon, we have attempted to explain in our further research.

4. Role of Warm-Blooded Animals in Dissemination of Botulism

The part played by animals in the distribution of botulism has not been finally explained to date.

However, the participation of certain warm-blooded animals in the dissemination of botulism does not provoke comment. Thus, at the present time it is known that botulism is found among domestic animals. The most sensitive to botulism toxin is the horse. The first to observe botulism in horses and mules in 1917 were Graham, Branker and Pontius (1918): forty mules and nine horses

were poisoned from pressed poor quality fodder that had been dirtied by chicken feces. The animals which were inoculated with anti-botulism serum remained healthy, whereas those which were not inoculated became sick.

Graham and Schwartze (1922) observed the illness of horses, a sickness very similar to botulism. According to their hypothesis, the disease was precipitated by poor quality hay or oats. Under bacteriological analysis, the bacillus of botulism was not isolated from either hay or water. The cadavers of horses that had died from this fodder were also subjected to bacteriological observation. From the spleen of one horse, a very toxigenous strain of the botulism stimulant type A was isolated. The toxin of this microbe was neutralized well by type A anti-toxin, derived from olives. The authors comment that the botulism sickness is encountered especially often among horses that feed on silo fodder.

The cases of botulism among horses in Canada are described by Mitchell (1922). In 1923, in the veterinary literature of England and the USA, a great discussion took place about the so-called "grass disease" among horses which was observed in Scotland and England. Paralysis at times takes place in horses with this disease which is very similar to botulism.

Certain authors consider this illness to be botulism; others claim that the mentioned disease of horses has not connection with botulism. The etiology of this illness has remained to date unexplained.

Tayler and Robinson (1926), during the disease of botulism among mules and horses in South Africa, discovered the cadavers of rodents in the feed boxes of animals. From the cadaver of one mouse, the authors isolated a very toxigenous microbe which

they named Clostridium Botulinum. An extract from the organs of this rat possessed very poisonous properties. It caused typical botulism among laboratory animals. Besides, this extract precipitated the disease of botulism in horses.

During the study of this microbe, Robinson (1930) classified it as the type Bac. Botulinus C. Tayler and Robinson (1930) specifically emphasize that botulism is encountered among domestic animals only in the event that the fodder is contaminated by the decomposing carcasses of rodents: mice, rats, rabbits. Later, the possibility of botulism in horses and mules was corroborated in the reports of Fulton (1929), Donatein and Lestocar (1930) as well as other authors who had observed botulism in mules and horses at Algiers.

In the Soviet Union, the first report about botulism affecting horses was oublished in 1932 by Konyshev and Gamaleya in the "Works of the Novocherkassk Veterinary Station" (1931-1934). The authors observed a botulism epidemic at 16 points in the Rostov province. The cause for the poisoning in 90 percent of the cases could be traced to the corn silo and also to the husks which had been stewed 4 - 6 days. A similar disease in horses was observed by Shostak and Lenshin (1936) in the Donets province, in the course of which they were able to isolate from the fodder the toxin of the botulism microbe.

In 1937 Dukalov observed the poisoning of horses from silos of 17 different farms in the Ordzhonikidze district. With the stopping of fodder from the silos, the disease among the horses also ceased. It is interestin that this same fodder was eaten by cows and pigs without any ill after-effects. As bacteriological tests showed, the poisoning of horses was caused by the toxin

of the botulism microbe. Very toxigenous cultures of the botulism bacillus were isolated from the silo and from the contents of the dead horse's stomach.

Bobashinskii and Polyakov (1938) reported on epidemics of botulism among horses. In one farm, nine horses died; in another - fourteen. One of these epidemics was verified bacteriologically. It is apparent that silos may present favorable conditions for the formation of toxin by the bacillus of botulism. It is possible that this is connected with the strong contamination of fodder by the soil when loading the silos. It is also necessary to ascertain that the cadavers of rodents do not fall into the silo (rats, mice). The formation of toxin by the stimulant of botulism in silos was proven through the research of Firsova and Pokhil (1935).

Graham and Schwartze (1921) earlier described an incident of botulism among cattle in certain of the North American states. Soon thereafter (1922) in Australia a similar disease was observed by Seddon which was accompanied by symptoms of paralysis in the medulla oblongata. He called it the Tasmanian Midland disease. This sickness, in its clinical symptoms, looked like botulism and took place in an acute as well as chronic form. Usually the disease commenced after the animals had eaten the remains of infected skeletons. The author was able to isolate a very toxigenous microbe from the decomposed bones of a cow that had died four months before. The microbe appeared to be very similar to the stimulant of botulism, discovered by Bengston, and it was designated as Bac. Botulinus C. In 1920, Tayler described the disease of botulism among cattle in South Africa which was known there under the name "lamsiekte." In Africa this sickness is encountered during the period of phosphorus starvation

among cattle. As a result of phosphorus deficiency in plants, the animals begin to eat manure, bones, and the cadavers of rodents. Bones at time contain a strong poison, causing illness, as was shown by Tayler experimentally. Only in 1927 was Tayler able to isolate a pure culture of the stimulant. This was a strongly toxigenous microbe, precipitating a typically botulism illness. The author named this microbe Clostridium Parabotulinum Bovis. Later on, Meier and Vainberg suggested a different name - Bac. Botulinus D.

In literature one also find reports on the disease of botulism among sheep. In the opinion of certain authors, this sickness is accompanied at times by a general paralysis and has been observed among sheep which had eaten the cadavers of rabbits. In these cadavers, the Bac. Botulinus B. was discovered.

The disease of botulism in cattle throughout the Soviet Union has not been described by anybody. Cattle apparently in general rarely become stricken with botulism which is explained by their low sensitivity to the toxin of botulism.

Still less affected by the toxin of this microbe are pigs. Kempner and Polyak (1897) described a case where a pig became sick with botulism. In the course of one of their experiments, they were able to isolate the bacillus of botulism from the pig three months after the disease. Pigs seldom are sick after eating spoiled canned goods. In the manure of these pigs, Burke discovered the bacillus of botulism. According to the observations of Easton and Meier (1924) the bacillus in certain instances is found in the intestines of pigs that are healthy. This precipitated the hypothesis about the length of time the botulism bacillus can be carried in the intestines of a pig. The latter, in such a case, can become the cause for disseminating botulism. However, this hypothesis still needs corroboration.

Botulism in chickens has been known for a long time. Dixon already in 1917 noted the similarity of botulism with "limberneck" in chickens. In the course of lengthy research, he was able to establish that limberneck in chickens is caused by poisoning from spoiled fodder which contained the toxin of botulism. Meier observed 125 epidemics of botulism among chickens, during which about 3,600 chickens died. In 30 cases, the botulism diagnosis was supported by toxicological and bacteriological tests. In the majority of cases, the illness was caused by the botulism bacillus of the A type, to which chickens are apparently sensitive. Hart (1920) described an epidemic of botulism, encompassing 634 chickens, which started after they had eaten from one can of spoiled beans.

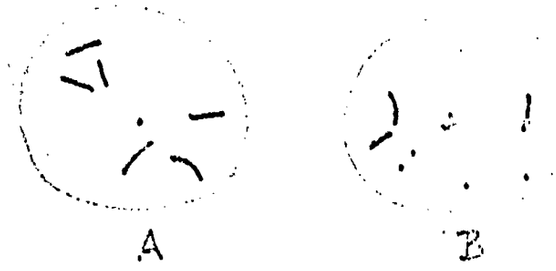
Saunders (1921) first noticed that the limberneck disease in chickens could be observed after they had eaten larvae Lucilla Caesar. In 1922 Bengston isolated Bac. Botulinus from larvae found in the cadaver of a chicken that had died from limberneck. This microbe produces a very strong toxin, similar in its properties to the toxins of other strains from the botulism microbe. After careful study, it was placed in the type Bac. Botulinus C.

In the salt flats (estuaries) of California, as well as along the banks of rivers, the western duck disease is widely distributed. The mass death of wild birds attracted the attention of researchers already in the 'nineties of the last century. For a long time, the cause for these strong epizootics among wild ducks remained unknown. In certain years during the migration period, from several tens of thousands to a million birds would die. Only as a result of systematic observation, conducted from 1914, was it possible to explain that the "western duck disease" was nothing else but botulism.

During the periods of a strong epizootic of botulism in ducks, Jiltner and Kooch (1930), Hohmeier (1930) and also Kallembach and Henderson (1934) were able to isolate Bac. Botulinus C in them. Apparently this microbe can remain in the organism of birds and not reveal itself. Thus, for example, Henderson (1933) discovered the bacillus of botulism in ten healthy ducks. Klambeck (1930) by means of inoculation with toxin and spores of the botulism bacillus, type C, proved that the "western duck disease" in California is botulism.

A great epizootic of botulism among ducks, from which over a million of the birds died, was observed by Kallembach and Henderson in 1932 and 1934 respectively. In certain places large areas of marshland, the banks of rivers and lakes were covered by the bodies of wild ducks.

The bacteriological research of the above mentioned authors, studying the epidemic of botulism in 1932, and also the works of Pullar (1934) established that the epidemic was caused by Bac. Botulinus C (illustration 1).



Illustr. 1. Stimulant of the Duck Disease

A - vegetative form of Bac. Botulinus C;

B - two bacilli of Bac. Botulinus C with subterminal spores.

The decease of botulism among birds is also encountered in marshlands that have a little standing water with particles of alkalized soil.

In separate parts of the marshlands, there are at times concentrations of fibrous water algae which hinder the movement of the water. In these places during hot days favorable conditions are created for decomposition, in the course of which an intensive absorption of oxygen takes place. This aids the development of the botulism bacillus C and the formation of a large quantity of toxin. A considerable role is also played in this process by the alkalization of water and soil.

Usually epidemics of botulism in birds are observed after hot days, in August and September. At first the toxin of Bac. Botulinus C was discovered in the organs of dead birds. With the assistance of biological tests and neutralization reactions upon pidgeons, it was established that the toxin of that microbe was present in water, dirt and in the larvae of flies. From 76 samples, taken on the shore of a lake, in 22 instances the toxin of botulism bacilli was found. In stagnant water around the cadavers of ducks, the toxin of botulism was also discobered. Living or dead larvae of flies, located on the decomposing bodies of ducks, contained toxin in large quantities. Certain of them were carried by the wind over a large area of water and caused the death of birds that ate them. The remains of these larvae were found in the stomachs of the dead birds. Consequently, the dead bodies of ducks, stricken by botulism, play a considerable role in the infection of separate parts of the marshlands. The toxin in the dirt of the marshlands, after the removal of the dead birds, is preserved up to 17 days.

Crystaline deposits of alkaline salts (potash, calcium, sodium, and others) on algae apparently help in the formation of toxin.

During the observation at these places, Henderson (1934) took 55 samples of dirt, remains of algae and other materials. He discovered in 47.3 percent of them Bac. Botulinus C. A strong botulism toxin was extracted from the remains of insects, algae and snails taken from locations where alkaline salts were deposited.

When stricken by botulism ducks are not capable of movement as a result of paralysis in their muscles. They remain sitting on one spot until they die or recover. The large percentage of recoveries among birds can apparently be explained by the insignificant amount of toxin that penetrated into their organisms.

According to the research of Academician E. N. Pavlovskii and his collaborators (1939, 1946), certain of the bacterial infections have a natural nucleus of infection. As the above mentioned data show, botulism in separate places on the earth apparently also has its natural nucleus. The natural nuclei of the botulism infection can be found in certain of the western states in the USA: in Oregon, in central and southern California, in Dakota and others. In individual places, due to the drying of the marshlands, the size of the infection nuclei has grown smaller. The source for the dissemination of the botulism infection appear to be wild birds. During a period of mass death among the birds, muskrats and other carnivorae also die from botulism because they eat the bird cadavers containing the toxin of botulism.

In connection with this fact that certain epidemics of botulism among domestic animals have been caused by contaminated fodder with the cadavers of rodents, it seemed of interest to explore the role of wild rodents in the dissemination of botulism. Burova,

Bukisova, Kats and Segal (1936) undertook and experimental study of this problem. They infected gray rats through the mouth with the bacilli of botulism, which had been relieved of toxin. Subsequently long range observation was carried on to establish the secretion of this microbe from the intestines of the animals. Similar tests were conducted also on chickens.

It was established that grey rats even on the 25-th and 46-th day, and chickens on the 110-th day, after their infection still secrete the bacillus of botulism. Experiments showed that chickens and rats are little sensitive to the toxin of botulism. In this connection, the authors came to the conclusion that animals which are anly a little sensitive to botulism toxin can be the source for the dissemination of botulism. Especially dangerous in this connection are chickens and rats. Being themselves unsensitive to infection, they can secrete the botulism microbe from their intestines for a long time and spread it in their surroundings.

The foregoing data from the literature on the subject shows the considerable role played by certain animals in the distribution of botulism. It can be accepted as established that wild birds, wild rodents, and the cartilaginous fish appear to be at times not only factors in this dissemination but also the causes for the outbreaks of botulism epidemics among people.

5. Grey Rats as a Factor in the Dissemination of the Botulism Agent in Enterprises Processing Food

The struggle against grey rats (mus decumanus) is very difficult. Appearing to be undemanding in their needs, they devour not only products of good quality but also any kind of scrap. When they eat cartilaginous fish, which may be infected with the spores of the botulism bacillus, rats can become carriers of this microbe for a

long time.

In order to explain the possibilities of this phenomenon, we conducted an observation of rats and their carrying of botulism bacilli. The procurement of rats was conducted by means of traps or with the assistance of specially trained dogs. A total of 50 rats were placed under observation. Specimens were taken from all animals from the heart, the liver, the spleen, the contents of the stomach and the right intestine. These were placed into two retorts with 200 cubic centimeters of Tarotstsi broth under a layer of vaseline. One of the retorts was heated 15 minutes at 80 degrees in a water basin, the other was not subject to heating.

On the 8 - 10th day in the thermostat at 37 degrees, a grown culture was obtained. Microscopic observation was instituted, and a preliminary bio-test for the presence of the toxin from the botulism bacillus was made. The culture was next purified by leading it through a high agar. After this, it was determined whether it belonged to an agent of botulism by means of studying the morphology, the neutralization reaction to the toxin, and the agglutination reaction.

Of the organs from the 50 observed rats, the microbe of botulism was isolated in 12 instances (table 7).

In nine cases, these were toxigenous strains of the A type, whereas in three cases - non toxigenous strains, of which one produced an agglutination with the serum from a type B botulism bacillus.

The microbe was found in the blood of the rat's heart twice, in the liver - seven times, in the/spleen - six times, in the stomach - five times, and in the right intestine - three times. The discovery of the microbe in the internal organs appeared to be clear proof of its ability to penetrate through the walls of the intestines into the parenchymatous organs. It is important to note that when wild

rats fed on cartilaginous fish, which had been infected by the spores of the botulism agent, an infection of the rats' organism took place in their natural conditions of existence.

Table 7. Carrying of Bac. Botulinus by Grey Rats

Number	Designation of rat	Organ from which secretion occured					Morphology	Toxigeno's	Neutral. reaction to toxin		Agglutination reaction	
		Heart	Liver	Spleen	Stomach	Right intestine			With ser'm A	With ser'm B	With ser'm A	With ser'm B
1	1	0	+	+	+	+	Bacilli and "Paketti"	+	+	0	+	0
2	4	0	ø	0	+	0	ibid.	+	+	0	+	0
3	15	0	+	0	0	+	ibid.	+	+	0	+	0
4	18	0	+	+	0	0	ibid.	+	+	0	+	0
5	20	0	+	+	0	0	ibid.	+	+	0	+	0
6	26	0	+	0	0	0	ibid.	+	+	0	+	0
7	27	+	0	0	0	0	ibid.	0	0	0	+	0
8	29	0	0	0	+	0	ibid.	0	0	0	+	0
9	34	0	+	+	+	0	ibid.	+	+	0	+	0
10	35	0	0	+	+	0	ibid.	0	0	0	0	+
11	37	+	0	+	0	+	ibid.	+	+	0	+	0
12	39	0	+	0	0	0	ibid.	+	+	0	+	0
Totals		2	7	6	5	3		9	9	0	11	1

NOTES + positive result from observation

- negative result

In consideration of the substantial degree of stability evidenced by rats with regard to the botulism toxin, it is possible to make the hypothesis that infection runs its course benignly in them. It is possible that the rats underwent a symptomless botulism infection, which ended harmlessly with a gradual removal of the microbe from the organism.

In our experiments, we set ourselves the task of explaining the possibility that a botulism infection can exist in animals without any symptoms.

Observations, conducted on grey rats, represent an aspect of considerable interest for a correct understanding of the pathogenesis of botulism infection. They show that spores, which have fallen into the intestines together with food products, lead to the infection of the organism where they grow and transform themselves into vegetative forms with the production of toxin. In this connection, the problem arises concerning the possibility of such a phenomenon in a human being after eating products contaminated by the spores of the botulism bacillus.

The infection of grey rats with the botulism bacillus could have taken place from the cartilaginous fish (the 'beluga,' 'osetr' and 'sevryuga' types of sturgeon), which the rats devour willingly. During the period of observation, there were instances of intake of cartilaginous fish containing the botulism microbe, which was established through bacteriological tests. In such rats, the microbe of botulism was secreted from the content of the right intestine. Consequently, in these animals the botulism agent can not only be maintained in the organs but also be secreted in the excrement. The data obtained undoubtedly corroborates the possibility that [illegible] botulism.

From this, a series of important measures flow which should be introduced at food processing enterprises. Rigid control is imperative, so that products contaminated with the microbe of botulism will not be stored in warehouses and refrigerators. The latter can then be utilized for the preservation of other products. Also the products which are already infected with the botulism microbe must be maintained in areas to which rats have no entry. The annihilation of grey rats in food enterprises should be compulsory. This measure appears to be one of the most important in the struggle against botulism, especially in fish factories.

The results, obtained by us in the study of the grey rat's role in the distribution of botulism, reaffirm us in the possibility for the agent of this infection to penetrate through the walls of the intestines into the internal organs in connection with the eating by animals of fish that is contaminated by spores.

PATHOGENESIS OF THE BOTULISM INFECTION

CHAPTER III

Insufficient attention has been given up to the present time to the study of the pathogenic properties of the botulism agent. Van Ermeng, in explaining the cause for poisoning at Ellentselen, obtained Bac. Botulinus from ham and studied its toxigenic properties. In the opinion of the author, the poisoning from ham took place exclusively as a result of the toxin's action; the infectious properties of the microbe played no role whatsoever. These conclusions were based primarily upon the fact that the organs and blood of the experimental animals, into which an extract from the ham was introduced, were free from microbes. Passages undertaken on animals in the search for the botulism agent were also unsuccessful, even after introduction of massive doses of the ham extract which contained a large quantity of spores.

All of these observations led Van Ermeng (1912) to the erronious conclusion that botulism was apparently an exogenic intoxication and not a toxic infection. In his view, the botulism bacillus appears as a "toxigenic saprophyte" which can not spread in an animal organism. In the work of Van Ermeng, all attention was directed toward an explanation of the results from the toxin action upon the organism of animals and the human being. The role of the microbe itself was not taken into consideration at all in this connection.

In studying the culture and biochemical properties of this microbe, Van Ermeng arrived at analogous results. The conditions for the cultivation of of the botulism bacillus, in his experiments, were completely non-typical for pathogenic microbes. The optimum

temperature for growth was found to be between 25 and 30 degrees; only at such a temperature did the microbe multiply well and produce a strong toxin. At a temperature of 37 to 38.5 degrees, in the cultures under observation, threads were seen to form. They looked long, thin, and in places fat. These threads soon passed into involute forms; in the process, only insignificant quantities of toxin were produced. On the basis of this research, Van Ermeng came to the conclusion that it was impossible for the botulism bacillus to multiply in the organism of animals and man at a temperature of warm blooded bodies.

Further observations of various authors provided contradictory results.

Studying the strains of this microbe, obtained from the excrement of a pig, Remer (1900) noted that they developed better at a temperature of 22 degrees. Schumacher (1913) obtained similar results upon cultivating the botulism bacillus from ham: it developed well at 18 degrees but much worse at 37 degrees. Dixon (1918) studied the cultures obtained from numerous epidemics and observed a good growth of this microbe and the formation of a very strong toxin at 20 to 30 degrees.

Grekhem and Bruckner (1919) reported that botulism cultures, isolated during poisoning from fodder, developed well only at temperatures between 22 and 25 degrees.

Tom, Edmondson and Jiltner (1919) observed a good growth of strains, isolated from asparagus, at 37 degrees. The most propitious for the growth of these cultures appeared to be a temperature of 35 degrees; under these conditions, a very strong toxin was produced.

Burke (1919), occupied with the study of the conditions for the development of various strains in the botulism bacillus, observed

a good development of certain strains at temperatures between 22 and 28 degrees; in others, multiplication took place more intensively at 37 degrees; lowering of the temperature stopped the development of the cultures. Toxin was produced by all strains equally well between 28 and 38 degrees. Similar results were obtained by Bitter (1919) and Landman (1904) during the growth of the microbes: their strains multiplied well only at 24 to 25 degrees, and under these conditions a very strong toxin was produced.

Other authors, studying the biochemical and culture properties of the botulism agent, received completely opposite results. Even in 1902, Matsushita reported that in his experiments cultures of botulism developed better at temperatures between 34 and 38 degrees and that under these conditions a stronger toxin was formed. Even at 45 degrees, the author observed a multiplication of bacteria. The most advantageous, in his opinion, was a temperature of 38 degrees.

The botulism bacillus, isolated by Leich (1912) from canned beans that had been the cause of poisoning, developed well only at 37 degrees. Armstrong, Story and Scott (1919) obtained at 37 degrees a more rapid multiplication and toxin formation by strains which had been isolated from plums that had caused poisoning. They noted that at room temperature, the growth was slowed down. Remer (1919) undertook a similar study of the conditions for toxin formation in 16 different strains of the botulism bacillus. He observed a good multiplication and a potent toxin formation at 37 degrees. Orr (1919) as well as Nevin (1921) on the basis of their research came to the conclusion that 37 degrees was the most advantageous temperature for the growth of the botulism microbes; under these

conditions, the microbe grows rapidly and produces a very strong toxin.

With the accumulation of large quantities of strains from the botulism agent, in the laboratories of different countries there began to appear more and more reports on the good growth of the strains and their toxin production at 37 degrees. In 1922 the work of Dubovski and Meier was published. The authors studied over a period of ten years more than 100 strains of Bac. Botulinus, isolated from many sources. The growth was conducted over different periods of time, under various conditions, and at different temperatures. The observations of the authors showed that the botulism microbe produces toxin well in the course of ten days at a temperature between 35 and 37 degrees. This temperature appeared to be the optimum for the formation of a strong botulism toxin. In such a way, on the basis of considerable material, it was found possible to produce toxin by the botulism bacillus at the temperature of warm-blooded beings. It can be deduced from the foregoing that the botulism microbe in its temperature requirements for toxin formation does not differ from other pathogenic anaerobes. This deduction was the stimulant toward further research on the pathogenic properties of the botulism bacillus.

Remer, Landman and Fersman in studying the pathogenesis of botulism obtained results analogous to the data of Van Ermeng. In their opinion, the spores of the botulism bacillus that had been introduced into the organism of animals even with lactic acid or agar in order to protect them from phagocytosis, could not be the cause of death from botulism in these animals. Shippen and also Graham and Bruckner (1919) infected animals with spores, that were free from toxin, under the skin or through the mouth. They

did not observe any botulism sickness in these animals. On this basis, the authors came to the conclusion that the botulism bacillus does not possess pathogenic properties, can not multiply or produce toxin in the organism of warm-blooded animals or man.

Armstrong, Story and Scott (1919) noted the difficulty in eliminating toxin by means of multiple washing of spores. After infecting guinea pigs with warmed spores, they observed no deaths of animals. Simultaneously, the spores that had been washed several times in a physiological solution caused the death of animals from botulism. Bullock and Cramer (1917) introduced into guinea pigs spores without toxin together with calcium chloride and observed the death of animals from botulism. This research was not corroborated by any other authors.

Dixon and Burke (1918) in the course of infecting animals internally discovered the botulism bacillus in their organs.

Tom, Edmondson and Jilter (1919), on the basis of experimental data, came to the conclusion that the botulism agent when free of toxin does not precipitate the symptoms of poisoning in animals, after the authors had introduced spores into the organism by different methods. In certain experiments over a period of 5 to 7 days, after the introduction of 180 to 192 million spores, the animals died from botulism. However, the authors considered the results from these experiments to be doubtful. The reason for their negative evaluation was the conviction that animals died from toxin which had remained after the washing or heating of the spores.

In 1921 Orr announced the possibility of obtaining the symptoms of botulism in animals after infecting them with spores that did not contain toxin. The animals became sick with botulism

as a consequence of introducing spores into the organism by various methods. Subsequently Orr (1922) in order to free the spores from toxin heated them over a period of one hour at 80 degrees. After a subcutaneous injection with massive doses of spores, free from toxin, guinea pigs and mice manifested symptoms of botulism. Certain animals died, while others only became sick. The author, in his experiments, showed the possibility of discovering the botulism bacillus in the organs of animals.

The microbe was found in the organs after infection with spores subcutaneously or through the mouth. The discovery was conducted at the moment of agony or immediately after death; in such a way, the post mortem multiplication of microbes was eliminated. As a result of his experiments, Orr did not claim the conclusions applicable to the human being. He claims that a case of botulism infection in man is rare, if at all occuring. In his opinion, it is difficult to imagine that man could consume with food such a quantity of spores necessary to induce botulism.

Similar results were obtained in their experiments by Geiger, Dixon and Meier (1922). Regardless of this fact, in analyzing the results of research conducted by them as well as by other authors, devoted to the epidemiology and pathogenesis of botulism, they voice the doubt that the spores of the botulism agent free from toxin are pathogenic for the human being.

The above mentioned authors claim that the possibility of poisoning the human being under ordinary circumstances by means of spores free from toxin receives little credence. From the utilization of low-quality raw products in food, there occurs botulism poisoning of people with fatal results. In the event [illegible] usually no symptoms of the illness

were observed.

Completely opposite observations were announced by Schoenholtz and Meier (1924). They fed guinea pigs heated canned peas and maize, which included large quantities of spores from the botulism bacillus, and the animals died from botulism. In theview of the authors, the specific products in the decomposition of of the canned goods can contribute to the illness of animals and human beings with botulism.

The results of research on the part of many authors are not being compared. In a considerable part of this work, similar conditions were not maintained in the conducted experiments. Often the result of research depends upon the method for computing the quantity of spores and vegetative cells in emulsions for the infection of animals. There are no accurate methods for this, and for this reason the subjective opinion of the researcher is of great significance.

Important also to take into consideration is the time element, i.e. the span between the moment the spores were freed from toxin and when they were introduced into the organism. Different results were obtained by infecting animals subcutaneously, through the mouth, and by other methods. It is difficult to evaluate correctly the many pieces of research also because no control had been established to check whether the spores were completely made free from toxin.

All of these factors apparently contributed to the stimulation of Coleman and Meier (1922) for their detailed study of the pathogenesis of botulism.

Guinea pigs were infected suboutaneously and through the mouth with spores of the botulism bacillus, which were carefully

washed an heated at 80 degrees in the course of an hour. Absolutely healthy animals were supplied with varying quantities of spores - from 42 million to 5 billion. It is necessary to note that at the given temperature, a certain number of spores will be destroyed; in order that there will remain a sufficient quantity of living spores, capable of development, they are introduced in such large quantities. The discovery of dead guinea pigs was conducted under conditions excluding the possibility of a laboratory contamination in the cultures from the organs. As a result, the guinea pigs that were infected with the spores became sick with botulism and died. They died at different times after becoming infected: the earliest time was 4 days, the latest being 14 to 15 days.

A considerable number of the animals that remained alive at the end of the experiment were killed after 10 to 27 days. In these animals, the botulism bacillus was found less often than in the animals that had died from the disease. The organs of the guinea pigs infected with spores subcutaneously were contaminated more intensively than those infected through the mouth. In the organs of the pigs that had died from botulism, the presence of toxin could not be established through a biological test. In the blood of the killed animals, without any symptoms of botulism, the botulism bacillus was not discovered. Most often, the microbe would plant itself in the liver and spleen. The authors explain this by means of the phagocytic properties of certain cells in these organs. When the infection took place subcutaneously, the agent was found in the liver of the dead animals in 92 percent of the cases, in the liver - in 77 percent, and in the brain - in 75 percent of the cases. With animals contaminated through the mouth, in the event of death from botulism, the microbe was found

in the liver in 80 percent, in the spleen - in 38 percent, and in the brain - in 54 percent of the cases.

Coleman and Meier observed in guinea pigs, that had been infected through the mouth, a long secretion of botulism bacilli with the excrement.

In order to prove that animals died from the toxin, formed in the organism, supplementary experiments were conducted. The spores without the toxin from the botulism agent were introduced into the tied slip knot v. jugularis of a rabbit and also into the front chamber of the eye. These experiments manifested the growth of spores and the accumulation of toxin in the organism of a rabbit.

On the basis of their experiments, the authors came to the following conclusions: 1) massive doses of spores Bac. Botulinus, free of toxin, possess pathogenic properties and when introduced into the organism of animals precipitate botulism sickness; 2) spores and vegetative cells are possessed of the ability to rapidly penetrate the tissues and the organs of animals; 3) spores that are free from toxin can transform into vegetative cells in the organism of animals and produce toxin.

The authors emphasize that all of these phenomena are possible only upon the introduction of large quantities of spores. They do not in any event transfer their data as being applicable to the contamination of human beings under ordinary circumstances. For this reason, they write: "Our results, showing the difficulty of infecting laboratory animals by other means than the introduction of massive doses of heated spores Bac. Botulinus, should not serve in any event as a criterium of what could occur in the human organism during the utilization of spores from this micro-organism in normal conditions. However, in the last analysis this is doubtful

because in order to precipitate an infection, a huge quantity of spores free from toxin is necessary.

This research was continued by Coleman (1923). The spores, washed in a physiological solution, were heated for one hour at 80 degrees in order to dissolve the toxin. Then they were placed into little pockets and sewn inside the stomach recess of animals (guinea pigs and rabbits). The spores in the pockets developed well and produced a very strong toxin which would not ultrafiltrate from the pocket over a period of ten days and longer. Food substances, in the opinion of the author, penetrated into the pockets from the organism. Bacterial cultures in the pockets did not contain spores even after the 22-nd day of being in the organism of animals. These experiments showed the possibility of germination on the part of spores and the production of botulism toxin in the organism of animals. In this connection, the author considers it of great importance that products contaminated with the spores from botulism bacilli never be used in food even after heating if the spores are not destroyed. In order to kill the spores, ordinary boiling for the preparation of food is insufficient.

In his second work, Coleman (1929) concerned himself with an explanation of the problem of the minimum quantity of spores from the botulism bacillus necessary to introduce into a necrotic particle of animal skin in order to cause sickness in it. As a preliminary, in the ourse of 3 to 22 hours a guinea pig received 0.6 to 0.8 ml of 10 percent formalin. Subsequently into the same place, varipus quantities of spores were introduced - from 25 million to 35 million. Their number was determined by means of planting large cultures on a high agar and by a simple totaling of the colonies.

In the necrotic nuclei the spores germinated, and in the strains from the nuclei there were many bacilli and filaments similar to spores that looked like cultures, and also toxin was produced. The animals died from botulism. In certain of the dead animals in the spleen many spores and bacilli were discovered, whereas in other organs such discoveries were rare. In tissues, taken from certain nuclei, a very strong toxin was present. An emulsion from them, even diluted 1 : 10,000 precipitated the death of mice. The control mice which were given an anti-botulism serum, remained alive. In two cases, the serum from the blood of guinea pigs that had died without botulism symptoms contained toxin in very large quantities. Such experiments were conducted on 14 rats, of which only one died. The author explains this by saying that he was unsuccessful in precipitating a sufficient necrosis of the tissue.

From these experiments it is possible to conclude that a condition supporting the growth and multiplication of botulism microbes was the amount of necrotic tissue in the organism of animals. The action of the toxin, in the opinion of Coleman, was cumulative.

In the research on pathogenesis of botulism, Sterin and Dek (1925) corroborated the results obtained by Coleman and Meier. These authors conducted observations with a careful totaling of the number of spores by means of a hematocytometer. In the course of an internal contamination of 20 rabbits with spores over different periods of time (from 36 hours to 8 days), five rabbits died. The animals in these experiments received from 90 million to 600 million spores from the botulism bacillus. Rabbits, infected with spores through the mouth, also became sick with botulism; in the event that they died, the botulism agent was sifted out of their organs: from the liver - 8[illegible] percent, from the spleen - 6[illegible] percent, and from the kidneys - 67

percent. Toxin was not discovered in the organism through a biological test.

All that Sterin and Dek conclude as proven on the basis of their experiments is the fatal result for animals after infection by spores of the botulism bacillus. However, they do not come to any conclusion on the possibility that this microbe will multiply in the organism of animals. White rats proved to be very stable toward botulism toxin and contamination from spores.

In the experiments of the above authors with the infection of animals by means of spores with calcium chloride and quinine, negative results were achieved. This corresponds to the data from earlier expriments by Hall and Davis (1923) who also came out with negative results for calcium chloride.

Sterin and Dek, in their experiments with the little pockets, obtained the same results as Coleman. The authors came to the conclusion that the spores from the botulism bacillus in the organism of animals can remain in a latent condition for a long time. Botulism in animals can develop only after large quantities of spores have been introduced. They consider it to be doubtful that spores not containing toxin could play a part in human botulism illness. The last conclusion is contradicted by their experiments.

A more valuable contribution toward the study of the botulism problem, and specifically its pathogenesis, was provided by Soviet science. A considerable service rendered by our native researchers appeared not only in the final corroboration through experiments on animals of the toxic-infectious properties of botulism but also the rational explanation of the botulism pathogenesis in man from the point of view of toxic-infectious nature of the process.

The first work on the pathogenesis of botulism in the USSR was published by Zelevinskaya (1935). She observed the death of animals from botulism after 10 to 38 days from the time massive doses (200 million) of spores were introduced under their skin. The spores had been freed from toxin by heating.

Guinea pigs, which had received a preliminary inter-muscular injection of 10 percent formalin and then in the same place an injection of spores, died from botulism after 3 to 6 days. In all cases with animals, Bac. Botulinus was sifted out from the organs.

After the internal infection with spores of rabbits that had been sick from coccidiosis, enteritis, or a pussy cold - they all died from botulism. The botulism agent was also sifted out from their organs. In the opinion of Zelevinskaya, her experiments corroborate the toxic-infectious pathogenesis of botulism.

Minervin (1936) undertook a study of the mechanism in the pathogenic functioning of the botulism bacillus. He infected guinea pigs through the mouth with a broth culture that had been given a preliminary heat treatment at 80 degrees during 20 minutes. Animals died in the presence of botulism symptoms; in two instances, it was possible to discover toxin in the organs of animals; in the remaining cases toxin was not found. The author explains this by claiming there was an insufficient content of toxin in the organs for finding it with the help of biological tests. In subsequent experiments by Minervin and Zilberman (1937), guinea pigs were contaminated through the mouth with heated cultures of the botulism bacillus. As a preliminary, the animals were fed milk with opium for weakening of the peristalsis; or the intestines were stimulated by bile which was introduced through a sounding borer. The animals died from botulism after 10 to 12 days, and botulism bacilli were sifted from the organs.

Minervin and Kotlyarevskaya (1937), in order to precipitate sickness with botulism in animals, applied the method of one-time sensibilization by means of small doses of botulism toxin. In the process of sensibilizing guinea pigs, the doses of botulism toxin were known to be unfatal (1/30, 1/50 of a fatal dose per guinea pig) and the authors obtained a more invariable botulism sickness during the infection with regard to small quantities of spores (from 6 million to 50 million). After the one-time introduction of a small dose of toxin into the guinea pigs, they remain sensibilized over a period of six days.

In their further research, Minervin and Kotlyarevskaya (1936) demontsrated the possibility of a non-specific sensibilization. It became apparent that the filtrates from toxigenous strains of B. Proteus, after introducing them subcutaneously and in certain instances enterally, precipitated sensibilization of guinea pigs to the toxin of botulism. It was also succeeded in sensibilizing them by filtrations from a putrid decomposition of chicken albumin. During the infection of non-specific sensibilized guinea pigs with spores from the botulism bacillus, a considerable percentage of the animals died from botulism.

The filtrates from a broth culture of Bac. Sporogenes in the experiments of Minervin and Kotlyarevskaya did not produce sensibilization. However, Burnos (1940) during a one-time contamination of guinea pigs with a heated culture of the botulism bacillus and Bac. Sporogenes observed the death of a considerable number of animals from botulism. She also succeeded in sensibilizing animals with filtrates of broth culture from hay bacillus; during the infiltration of animals by the heated culture from the botulism bacillus, they became sick with botulism.

As was indicated above, the organs of animals that had died from experimental botulism infection usually did not yield any toxin. Only Orr (1921) with the aid of a precipitation reaction was able to establish the presence of toxin in the organs.

The work of Minervin and Kotlyarevskaya (1937), devoted to the study of this problem, occupies a special place. The authors conducted a preliminary sensibilization of guinea pigs with the help of small doses of botulism toxin; then, the animals were infected with spores from this microbe that was free of toxin. The guinea pigs died from botulism in a large percentage; in the organs and tissues of these animals, by means of a biological test, it was simple to discover toxin. The presence of toxin was established in the urine for 86 percent, in the blood for 53 percent, in the bile for 50 percent, in the thin and fat intestines for 50 percent, in the liver and brain for 41 percent, in the spleen for 9 percent, and in the mesentery for 6 percent of the cases. During the introduction into guinea pigs of comparatively large quantities of one botulism toxin, the authors were successful in discovering it in the organs only rarely.

After the publication by us in 1937 of the work on non-symptom botulism infection, Minervin and Kotlyarevskaya in their research during 1939 also came to the conclusion on the possibility of obtaining latent forms of botulism in experimental animals. The authors made the hypothesis that the microbes of botulism, passing through the walls of the intestines into the internal organs, can produce latent nuclei there that would provide a dozing infection.

Gryanko (1941) came up with interesting data. During the infection of rabbits, guinea pigs and mice with large quantities of spores from the botulism bacillus (several hundreds of millions)

...s had been freed from toxin, the animals became sick and died from botulism. Applying the method of a preliminary sensibilization on animals with small doses of toxin and a subsequent contamination of them with small doses of spores, Gryanko obtained negative results: the animals did not die from botulism. Not knowing all of the details, which are of considerable importance in the arrangement of scientific experiments, it is difficult to explain the reason for the negative results obtained by the authors.

Gryanko, as well as other authors, in the event of death of the animals from botulism sifted the botulin bacillus from their organs. The author of the experiments considered that the spores of the botulism agent did not develop in the organs but degenerated.

At the present time, a large quantity of clinical and bacteriological data is available which support the toxic-infectious character of botulism's pathogenesis. This data was obtained in the course of observing those sick with botulism and during the bacteriological study of human organs from persons who died of botulism. Certain of these facts corroborate in full the observations reported from experimental work.

The possibility of developing latent forms of botulism in the human being is supported by cases of lengthy incubation periods, which have been known for a long time but to which no special importance was attached until the capability of the botulism bacillus to multiply itself in the organism of animals was established. With the establishment of this fact, cases of botulism with a long incubation period have been carefully studied.

Even in 1914, Wilbub and Ophules reported a case of botulism in a human being with an incubation period of 4 to 5 days. Meier (1928) cites in his work the reports of different authors on cases

of botulism sickness in which the incubation period attained 8 and even 14 days after the eating of infected food. Guertl and Gross (1934) described two instances of botulism with a slow development of the illness, beginning 3 to 4 days after contaminated food had been partaken of several times.

A botulism epidemic, with a slow development of symptoms and a very lengthy period of incubation, was described by Keller (1934) and pertained to 8 persons. Schtrom (1934) observed an instance of botulism in Norway having an incubation period of 2 to 3 days, after which the patients were afflicted by paralysis of the nervous system. It is interesting to note that one of the patients had a recidivation after 2 months.

Nikolenko and Kamenskaya (1936), having observed 306 cases of botulism, note that in 34 of the patients the incubation period was up to 5 days and more but that in some it came up to 13 days. Such a length for the incubation period can be explained only by the toxic-infectious origin of this disease. Koritskii (1937), after studying the clinical records of 260 botulism cases, notes that in 30 percent of them the incubation period lasted from 2 to 9 days.

Slutskii, Govseev and Bossin (1934) and also Changli-Chaikin (1937) have mentioned that some persons ill with botulism have a higher temperature. The authors explain this on the basis of the toxic-infectious pathogenesis of the disease.

In 1930 at the city of Sumy, a botulism epidemic occured that was caused by the flesh of dried sturgeon. The epidemic was observed by Burova, Mitelman and Nasledysheva (1935). Sixteen persons were affected. The toxin was discovered in the fish and also in the organs and blood of the patients. From the organs of the cadavers,

sec. Botulinus A was isolated. This epidemic of botulism was also characterized by a slow development of the disease. In some of the patients, the symptoms of botulism appeared on the second and third day after the consumption of the poisonous food. At first, intestinal phenomena made their appearance: heartburn, vomiting, strong formation of gas in the stomach, effervescent masses of vomit. The authors explain this by the multiplication of botulism bacilli in the stomach, during which as is known considerable gas is formed. These phenomena in certain of the patients were observed over a period of 1½ days after consumption of the dried sturgeon meat that had been contaminated with the botulism bacillus.

In this work, the authors describe the botulism epidemic at Kharkov in 1932 that was caused by salted sturgeon. Eighteen persons became ill. The agent of botulism was isolated from the organs of the dead persons. On the basis of their observations, the authors come to the following conclusion: "From a comparison of the clinical records on the development of the disease and the bacteriological data, it follows that in the described instances we were dealing not only with the intoxication process but also undoubtedly with the infection process."

In our opinion, these data on the length of the incubation period in a human case of botulism sickness can serve as the basis for a hypothesis on the role of small toxin doses from the botulism bacillus which are produced during the incubation period. The toxin is formed in the organism of a human being or an animal and gradually precipitates poisoning.

Interesting facts were discovered during the study of material taken from persons ill with botulism.

Friedman and Lorber (1937) conducted an observation of 36 persons ill with botulism and 36 others who had recovered in order to ascertain the content of the botulism agent in the intestines. They also checked for the presence of botulism toxin in the blood (48 patients), the spinal cord fluid (22 patients), and urine (35 patients).

During the research on excrement, they were able to isolate a toxigenous culture of the botulism bacillus in three cases on the 10th, 11th and 12th day from the time of poisoning. All patients, in whose excrement the microbe was found, were observed to have a heavy form of botulism. In 30 tests of the excrement from persons who had recovered from botulism, the results obtained were negative.

After observing the urine of 30 patients, Friedman and Lorber established the presence of the toxin in the urine of three patients on the 23rd and 32nd day after commencement of the sickness. In one of these instances, the culture of the botulism bacillus was also isolated from the excrement.

In two persons ill with a severe form of botulism, prior to treatment with serum, the authors discovered toxin in their blood on the second and third day from the time of poisoning. In research on the blood of 42 patients treated with serum, in two of the sick persons the toxin was found on the 2nd, 6th and 8th day of the illness. In both cases, it was a serious form of botulism.

The conducted observations leave no doubt as to the toxic-infectious nature of the pathogenesis of botulism. If this were to be excluded, then it would be completely impossible to explain

the origin of toxin on the 6th and 8th day of the sickness in blood and on the 23rd and 32nd day in the urine of patients, who had until that time been treated with anti-botulism serum up to 300 ml. internally and subcutaneously.

The appearance of the toxin in the blood and urine over such long periods of time, while the patient was being treated with serum, can be explained only through the multiplication of the botulism bacillus in the patient's organism, which accompanies the production of toxin and its entry into the blood with subsequent secretion of the urine.

In foreign literature, there have also been reports about the presence of toxin in the urine, excrement and blood of oersons ill with botulism.

In 1921, Meier and Geiger discovered two cases where the botulism bacillus was in the excrement of patients on the 6th and 11th day after the infection started. Some instances, where the botulism bacillus was secreted with the urine, are reported by Grekhem and Barger (1921). Kob discovered toxin in the serum of an infant on the 9th day of the illness. Zamerau and Noak (1919) found toxin in the blood of patients on the 4th, 6th, 9th, 16th and 25th day of the sickness.

There are also the reports of Dek and Buuda (1928), who introduced toxin into the stomach through a sounding borer of nine monkeys: in six of the monkeys, toxin was discovered in the blood.

In the spinal cord fluid of 22 patients, Friedman and Lorber (1937) did not find any toxin. The hematoencephalic barrier appeared to be insurmountable for the botulism toxin.

Data on the experimental study of this problem are given below.

During the current period, a considerable number of reports have been published on the results of bacteriological observations of human and animal cadavers caused by botulism. The first was Van Ermeng who isolated the botulism bacillus from the spleen, stomach and fat intestines of a human cadaver. He explained his discovery by the penetration of the microbe into the organs after the death of the patient. This explanation corresponded to his conception of the pathogenesis of botulism.

Dubovskaya and Meier (1922) undertook bacteriological tests of human organs of persons who had died from botulism, In two instances, they found the botulism bacillus. One of the times the microbe was secreted from the spleen and from the wall of the thin intestine; in the second case, from the brain. The toxin in the organs was not discovered. The authors also found the botulism bacillus in the spleen, liver and intestines of birds that had died from botulism. In certain instances, the organs of the birds contained botulism toxin. After the dissection of two horses and two cows that had died from botulism, a botulism culture was isolated from the liver and the lymphatic glands.

Shapiro and Nikolenko (1937) studied 21 human cadavers for the adsorption of toxin by parenchymatous organs and also the length of time it was found in the alimentary canal. In order to avoid the penetration of the microbe into the organs and the formation of toxin after death, the dissection of the cadavers took place over a period of several hours and at night.

The authors emphasize that toxin was discovered in the central nervous system alone during an incubation period of from one to three 24-hour units.

Attention is drawn to the fact that no toxin was found either in the stomach or thin intestines of the deceased during the first four hours after they had partaken of the poisonous food. Most often it was possible to discover toxin in the organs of dead persons, if they had been ill not less than four days from the time theyx had eaten the infected food. Toxin was found in the main part of the brain up to the seventh day, in the blood to the ninth or even 11-th day, and in the thin intestines to the 11-th day from the moment that the poisonous product was used. The presence of the toxin was established: in the spleen for three of the 15 deceased, in the liver for two among 10, in the bone marrow for three in nine, in the cephalic and spinal cord for five out of nine, in the mesenteric lymphatic glands for two in five, in the blood for two out of eight cases. Toxin was discovered in the stomach of two corpses out of six, but in the colons of all six cadavers. It was found to exist in the thin intestines of nine cases out of fourteen.

The existence of cultures from the botulism bacillus in the organs of these cadavers was also very considerable. Cultures of this microbe were obtained in four cases when planting took place from the mesentery of four corpses; in five cases out of ten from the spleen, in four out of four from the cephalic and spinal cord, in three out of five from the bone marrow, in one out of three from

the liver, in three out of seven from the stomach, in 13 out of 19 from the thin intestines, and in eight out of nine cases from the colons.

Thus, it was established by examining the organs of persons who had died from botulism that the stimulant had contaminated almost all of the organs. The contamination of the organs undoubtedly took place also during the life of the victims, since dissection of the corpses was conducted very soon after death. The presence of the botulism microbe in the living organs is explained by the authors as being due to the presence of toxin in many of the organs of cadavers examined by them.

On the basis of the foregoing experimental and clinical data, it seems to us that all objections against the possibility of the botulism stimulant penetrating through the walls of the intestines into the parenchymatous organs are surmounted. This has been proven through experimental research and bacteriological observation of the corpses of persons who died from botulism. The possibility has been established that toxin of the botulism bacillus can spread and develop in the organism of experimental animals.

Clinical observations provide the basis for the hypothesis that the spreading of this microbe and the production of toxin take place also in the organism of man, when he is ill with botulism. This is indicated by the fact that toxin was discovered in the organism over long periods after the illness began.

The presence of toxin in the organism was also established in patients who were being treated with an anti-botulism serum. Apart from this, in many instances it was observed that the course of the botulism illness possessed a lingering character.

In such a manner, the above mentioned materials do not leave room for the slightest doubt in the toxic-infectious character of botulism's pathogenesis.

Incidentally, until very recent times many scientists adhered to the opinions of Van Ermeng. The latter, as is well known, considered that the botulism microbe was not pathogenic, that it can not develop in the organism of animals or human beings and cause the illness through poisoning by the toxin which had developed inside of the organism in the food product. Unfortunately, even in the present time this position is accepted by many leaders in the field of medical microbiology and infectious deseases (Topli and Wilson, etc.).

1. The Provocation of Clinical Phenomena in Symptomless Botulinic Infections among Mice.

Currently, there is no doubt as to the possibility of obtaining experimental botulism in animals that have been infected with spores. A necessary condition for attaining this appears to be the contamination with a large quantity of spores from which the toxin has been eliminated. When contaminated with small doses of spores, the animals do not die from botulism. The quantity of spores needed for the development of the desease depends upon the method of their

introduction, the toxigenousness of the type used, and the ability of the botulism stimulant to multiply in the organism. Almost all of the experiments described in the literature on the subject were set up with animals that had been infected by a large quantity of spores from the botulism microbe, coming to several milliards and even billions in one milliliter. For this reason we set ourselves the goal of studying the pathogenesis of the botulinic infection in animals contaminated by small quantities of spores, which would not precipitate their death from botulism. Death of the animals was also excluded because of toxin which could appear in the organism as a result of dispersal by the spores. Apart from this, our experiments approximated to a certain degree the natural conditions of infection by the spores of animals and man, especially when experimental animals were infected through the mouth with non-lethal doses.

Easton and Meier (1924) announced their hypothesis on the complete harmlessness of small quantities of spores for man and animals. In this view, small quantities of spores are not held inside the organism but are mechanically excreted through the intestines of the human being. This same point of view was adhered to by Burova (1939).

In our ppinion, the hypothesis of Easton and Meier on the mechanical passing of small quantities of spores through the intestines of man and animals is contradicted by their own research (1921-1922) and the data of other authors. Apart from this, the

absence of apparent clinical symptoms of botulism during the employment of products infected with spores after inspection still does not signify complete harmlessness in general. Everything depends upon the quantity of products, degree of their infection by spores, the toxigenousness of the botulism microbe, and the resistance of the human organism. Parts of the product, which has been slightly contaminated by spores, are completely harmless; however, separate parts may be strongly planted with spores. The harmlessness of the latter, when used in food, gave rise to the doubt which in turn served as the reason for experimental research.

In our experimental animals, contaminated by sub-lethal doses of spores from the botulism bacillus, life was maintained over long periods of time without manifestation of any symptoms of illness. This fact brought us around to the thought that as a result of introducing spores into the organism of animals, a symptomless botulinic infection developed. In order to establish the correctness of this hypothesis, we applied the methods of provocation and utilized the surgical trauma as a provoking factor.

Observation of mice was conducted, and various quantities of spores from types A and B of the botulism microbe was introduced subcutaneously. During 10, 30, and 45 days in the mice the spleen or bud receded; in certain instances laparotomy or amputation of the legs were conducted. In cases of splenectomy, after 20 to 24 hours from the time of the operation, the animals underwent anti internal blockade with sugar of iron at 0.5 or subcutaneously with

trypan blue at 0.05 per one gramme of weight. Those mice were considered to have died from botulism, when they provided a characteristic clinical chart and a positive result from their organs - liver, heart, and also the place where the spores were introduced - in the form of cultures. The cultures were made in a Tarocci habitat under a layer of vaseline. The anaerobic cultures, morphologically similar to the botulism microbe, provided a neutralization reaction to the toxin of the anti-botulinic ~~xxxx~~ serum. The surgical trauma was applied in order to explain the length of time microbes can exist in the organism of animals and not lose their property of precipitating the botulism sickness.

The computation of the quantity of spores in one milliliter was accomplished by comparing the viscosity of the spore suspension in a physiological solution with standard B. ~~xxxx~~ salt. In size, the intestinal bacillus is much smaller than the botulinic microbe. Apart from this, the suspension of spores taken from a fluid habitat always contains an admixture of particles from the habitat. Because of this, the quantity of spores introduced during the infection process was as a matter of fact considerably smaller than that shown by the standard. In planting various cultures of spores prepared from a suspension according to specification in two milliards, with a high agar in order to obtain separate colonies, not more than 50 to 70 million spores with life properties in one milliliter appeared. The quantity of 500 million spores portrayed by us in the tables corresponds to 12 - 15 million spores with living properties.

During the first series of experiments, the mice were infected through the suspension of spores from the botulism microbe with the simultaneous introduction of 0.25 milliliter of anti-botulinic serum for neutralization of the toxin. In the remaining experiments the quantity of spores was decreased. Death of mice in the first series of experiments from botulism to splenectomy was almost non-existent: in the course of three tests, four mice out of 98 died from botulism. In all of the experiments of the first series, splenectomy and blockade were conducted on 94 mice; of these, 65 (69%) died from botulism. On the other hand, of the 106 control mice on which no splenectomy took place, three (2.9%) died.

It is apparent from the tests in the first series that with a decrease in the quantity of subcutaneously introduced spores from the botulism stimulant also decreases the percentage of casualties in mice after splenectomy. In tests No. 4 through 11, in which the dose of infectious material was decreased from 375 million to 2.5 thousand (according to the specification in two milliards), the deaths among the animals correspondingly dropped from 60 to 20%.

In the second series of tests the mice were infected with a suspension of spores from the botulism microbe that had been washed five times in a physiological solution and heated for one hour at a temperature of 80 degrees in order to eliminate the toxin. During the course of four tests in the second series, the animals were infected by 500 million spores (according to the specification of two milliards B. coli salit); in five tests the mice were contaminated by the

fifth washing fluid that had been poured off from the spores after centrifugal action at 3,000 revolutions over a period of 30 minutes. Splenectomy was conducted in all experiments of this series on the tenth day. Before the operation in one of the tests of the second series, two mice died from botulism; in a second test, three mice died. In the remaining experiments, no deaths of mice prior to the operations were observed. Of the 56 mice in the second series of tests, after splenectomy and blockade, 48 animals (85.7%) died from botulism; among the 47 control mice that did not undergo splenectomy, only two mice (4.2%) died.

In the third series of tests, the mice were infected by five milliards of spores (according to the standard of two milliard B. coli salt) that had been washed and heated to 80 degrees for one hour. The mice underwent splenectomy, neurectomy, laparotomy, and amputation of the legs. The desease was obtained with splenectomy in 100%, with neurectomy in 100%, with laparotomy in 50%, and with amputation of the legs in 27% of the cases.

The experiments in the third series show that a heavy surgical trauma, weakening the animal's organism, leads to provocation of clinical phenomena during a symptomless infection of mice with the botulism microbe. In all series of tests, mice died from botulism on the second or third day after the operation, and only an inconsiderable number of them died on the fifth or sixth day.

It follows from the above described experiments that the spores of the botulism bacillus, introduced subcutaneously into the organism of mice, precipitate in them a symptomless infection. The cause for

this appears to be the weak penetration of the organism by the microbe spores. The organism is, therefore, under the influence of the toxin produced by the microbe. It creates an anti-toxin, as a result of which at certain times the phenomena cpnnected with the development of botulism do not take place in the animal. However, as soon as the normal condition of the animal organism is destroyed under the influence of surgical trauma, the symptomless infection changes into one expressed in terms of a clinical illness. Botulism quickly develops in the animal and has a deadly result. The transition of the symptomless infection into an obvious sickness is explained probably in the following way: the production of anti-toxin is slowed down while the organism is weakened; the formation of toxin can be even increased in its rate of production, especially in the destroyed areas where the tissues have been necrotized.

In summation of the results from these experiments, conducted upon 297 tested and 188 control mice, it is possible to accept as established that mice undergoing surgical trauma pass from a symptomless botulinic infection into a clinical and open desease phase which precipitates rapid death from botulism.

By means of these observations, it was established that the botulism stimulant after being introduced into the organism of mice may be preserved up to 45 days and not lose its property of provoking desease. This fact was first established by us in 1937. The development of the illness in animals after the operation, in our opinion, took place in connection with the formation of large particles of dead tissue in the area of the wound. In the case when

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the alimentary canal of the mice. The infected animals remained under observation over a period of ten or more days. Then, the splectonomy was effected and in one test a neurectomy.

...e surgical trauma was applied in order to explain whether the spores penetrate through the walls of the intestines and how long the microbes can remain in the parenchymatous organs without losing their property of causing the botulism deseqse.

Computation of the quantity of spores in one milliliter was accomplished the same way as in the preceding experiments. The number 50 to 70 million vibble spores, shown on the tables, corresponds to the viscosity of the suspension for specification B. coli salt in two milliards.

In the first series of tests the mice were contaminated by 12 to 15 million and 25 to 35 million viable spores of the botulism microbe. The splenectomy was conducted on the tenth and 13th day after the spores had been introduced.

Prior to the operation of 160 mice six died, and in four of the latter cases the botulism microbe was isolated from their organs. After the operation from among 77 mice under observation 17 (22%) died from botulism. The percentage of death among the mice contaminated with 12 to 15 million spores averaged between 7.6% and 14.2%; among the mice infected with 25 to 35 million, death affected from 20% to 50%. Among the control animals, which were not operated upon, over the same period only three (3.9%) died.

In the second series of experiments the animals were infected with 50 to 70 million spores, which were introduced twice in

separate doses of 25 to 35 million each at an interval of four to six days. Prior to the operation, out of 103 mice only three (3%) died. The operation was conducted ten days after the first contamination. Of the 49 animals operated on, 17 (33%) died from botulism; among the 51 control mice, no death was observed.

In the third series of tests, the infection of mice was undertaken through 100 to 200 million viable spores. The latter were introduced three times, with 50 to 70 million each time, at intervals of five to seven days. A total of 113 mice were infected. Before the operation, six animals (5.3%) died of botulism. The operation was undertaken on the 15th, 17th, 18th, 76th, and 92nd day after the first contamination. In the course of the first three tests in this series, between 70% and 100% of the animals died; in the last two tests, this dropped to between 20% and 50%; in one experiment, not one animal died.

On the whole among 51 mice that underwent splenectomy, 36 (70.6%) died from botulism. Among the 56 animals in the control group, which were not operated upon, during the same period of time five mice (10.9%) died.

In the fourth series of tests the animals were infected with 100 to 200 million spores and were submitted to neurectomy. Under the influence of this operation, a provocation to infection was obtained on 80.5% of the animals.

The data received is compiled on Table 8.

Table 8. Influence of Surgical Trauma upon Symptomless Botulinic Infection among Mice after Contamination through the Mouth

Number in test series	Number of experiments	Quantity of spores introduced through the mouth of mice (in millions)	Number of infected mice	Died before operation	Percentage	Day of splenectomy after the infection	Day of neurectomy after the infection	Experiment: Number of mice operated on	Experiment: Died from botulism	Experiment: Percentage	Bac.botulinus in: Blood	Liver	Brain	Intestines	Spleen	Kidney	Control: Number of mice	Control: Died from botulism	Control: Percentage
1	6	12-35	160	6	3.7	10-13	-	77	17	22	5	3	7	8	13	0	77	3	3.9
2	6	50-70	103	3	3.0	10	-	49	17	33	5	13	5	0	0	0	51	0	0
3	6	100-200	113	6	5.3	15-92	-	51	36	70.6	2	20	20	18	21	0	56	5	10.9
4	3	100-200	79	4	5.7	-	15-18	35	30	80.5	5	13	11	14	0	19	45	5	11.0
Totals	-	-	455	19	4.2	-	-	212	100	47.1	17	49	43	40	34	19	229	13	5.6

The results of the four series of experiments show that, after infecting mice through the mouth with sub-lethal doses of spores from the botulism microbe, the animals develop a symptomless infection which can be transformed into a clinically expressed infection with the aid of surgical trauma. The possibility of provoking the latter depends upon the dose of spores at the time of contamination: the higher the dose of spores when infecting through the mouth, the higher the percentage of death among the animals from botulism after the operation.

Thus, when 12-15 million and 25-35 million viable spores were used during the infection process, the mortality of mice from botulism after the operation was equal to 22 percent; when 50-70 million were applied, it was 33 percent; when 100-200 million were given, the percentage was 70.6; whereas in certain experiments 100 percent of the operated mice died.

During the last three tests in the third series, the percentage of deaths from botulism was not very high (0-20-50). This can be explained only by the fact that the operation was conducted after a very long period of time had elapsed since the operation - on the 76th and 92nd day. The experimental and control mice, which had remained alive after the operation, were killed about four months after their infection. In the cultures taken from their organs, the botulism stimulant was not discovered, whereas at the time of operation the microbe had been isolated from the spleen of many animals. Apparently during the process of continuing bacillus carrying, there takes place a progressive freeing of the animal organism from the infecting agent. This process is probably connected with the production of immunity and the annihilation of the botulism microbe in the organism of the mice. It is possible to assume that the botulinic symptomless infection leads to the production of immunity among animals and human beings.

After the operation, the animals in all experiments were placed under observation for a period of 10-15 days. Death from botulism took place in the majority of cases on the 3rd-5th day

after the operation. The culture was made from the organs and blood of the mice in a Tarocci broth. During 6-8 days the cultures remained in the anaerostat and in an atmosphere of nitrogen. Then, computation of the cultures that had grown was undertaken by means of studying their morphology and also by means of a reaction neutralizing the toxin with the aid of an anti-botulinic serum.

The culture from the organism of mice was found to be very strong. In the specimens from the spleen, taken at the time of the operation, durin the first series of tests the microbe of botulism was discovered in 76.4 percent of the cases; during the third series, in 63.3 percent of the cases. Most often of all, the botulism bacillus was found in the liver - 49 percent; in the cephalic part of the brain - 43 percent; and in the intestines - 40 percent (see Table 8).

Subsequently, the spores of the botulism stimulant after having been introduced through the mouth penetrate the walls of the intestines and colonize the organs of animals. We were unable to determine the relationship between the intensity of this colonization in the animal organs and the dose of spores introduced into the body.

Many researchers studying the pathogenesis of the botulinic infection unsuccessfully have attempted to discover, with the help of biological experiments, the toxin of the botulism microbe in the organs of animals that have died from experimental botulism. In the literature on the subject, there are only two examples of research where the authors announce that they have succeeded in find-

ing toxin in the organs of animals deceased from botulism. One of these was the American Orr (1921) who discovered the toxin in the organs of animals by means of a precipitation reaction. The other was accomplished by Minervin and Kotlyarevski (1937) who, in order to obtain experimental botulism in guinea pigs, applied a very interesting method for a preventive specific sensibilization by means of small doses of botulinic toxin. In the organs of these guinea pigs, the authors established the presence of toxin from the botulism bacillus, with the aid of a biological experiment.

We also made an attempt to discover toxin in the organs of mice at the moment of their death from botulism. In experiment No. 2 (third series) nine mice were taken in the moment of agony just before death; this included the possibility that toxin was formed in their organs after death. In these animals, a clear picture of botulism was observed. They lay motionless with matted fur and heavy breathing; the diaphragm was paralyzed which caused a stretching out of the body (wasp-like waist). The intestines and liver of these animals were carefully comminuted in a porcelain mortar, after which the suspension of the organs was left for an hour on the table at room temperature in order to extract the toxin. Then the fluid underwent the centrifugal process and a biological test was made of it. One mouse received 0.5 milliliter of the fluid with 0.25 milliliter of normal horse serum; a second mouse was given the same quantity of fluid with anti-botulinic serum. Both mice remained alive. By these means, toxin from the botulism mic-

robe was not discovered in the organs of mice at the moment of their death from botulism, with the aid of a biological test.

The failure in the attempt to find toxin in the organs of mice can be explained in the following way: the toxin, progressively being formed by the botulism microbes, quickly united with the cells of the organs and there was not enough of it in a free condition for discovery through a biological experiment. This problem needs further study.

At this stage, the observation was set up in four series of tests in the course of which 212 mice underwent surgical trauma and 229 mice served as the control group and were not operated.

It was established that, upon contamination through the mouth with sub-lethal doses of spores, the mice developed a symptomless infection which could be transformed into a clinical and visible one by means of a surgical trauma. The spores of the botulism bacillus penetrate through the walls of the intestines, and in such a way the colonization by it of the organs takes place. The intensity of the colonization of organs does not depend upon the quantity of spores introduced at the time of contamination. At the moment of the mouses death from botulism, toxin was not found in its organs.

The completed observation shows that the hypothesis of Easton and Meier (1924) on the complete harmlessness of the spores from the botulism microbe which, according to their opinion, mechanically pass through the stomach-intestinal tract - is completely without foundation.

Botulism appears to be not only an intoxication but also and infection. Most often poisoning of the human being and animals takes place by the toxin which has formed inside of the organism in food products. However, it is also possible for the microbes to multiply and the toxin to form in the organs and the tissues which facilitates the development of the desease.

3. Infectious Properties of the Botulism Stimulant

Under natural circumstances, the human being and animals sometimes eat various products containing a considerable quantity of spores from the bacillus of botulism. If the product has no toxin, then the visible desease with a sufficiently expressed clinical picture apparently develops under such conditions very rarely. As has been shown above, certain researchers completely discarded the possibility of the botulism desease from the use of products containing only the spores.

We set ourselves the task of explaining the infectious role of spores from the botulism microbe when it has entered the organism of animals through the mouth together with the botulinic toxin. In this connection, it was important to establish whether the animals would come down with botulism if they would receive together with the spores less than a lethal dose of the toxin. Under such circumstances the botulism desease, it would seem, should not take place. However, as we already know from a survey of experimental data and literature on the subject, the spores penetrate from the intestines into all organs and can manifest their infectious

properties: multiply, germinate into vegetative forms, produce toxin. Under such circumstances, in the event that spores enter the organism through the mouth together with a small quantity of toxin, the animals should die from botulism.

For the purpose of explaining this problem, experiments were undertaken on mice and guinea pigs. The animals were infected by spores together with a small quantity of toxin through the mouth, with the aid of a metal pipe. As a precautionary measure, a lethal dose of dry botulinic toxin was titrated for the mice when introducing it through the mouth; it was found to be the equivalent of 0.0001 gramme. Two series of tests were set up.

The first series was conducted on mice. In order to infect the animals, seven to ten day cultures from the botulism microbe "A" were used. These contained a quantity of spores in large measure. The spores were washed several times in a physiological solution and heated at 80 degrees for one hour to eliminate the toxin. After this, the quantity of spores was computed according to the specification B. ~~salt~~ coli. As has been mentioned already in the preceding, in reality the quantity of live spores was much smaller than that on the specification.

In the first group of tests, there were 112 mice which were given from 25 million to 70 million spores and a one-third of a lethal dose of botulinic toxin. During these experiments, between 52 percent and 84.7 percent of the mice died.

At the same time among the control group of 48 animals, infected with the same amount of spores alone, from four to eight percent died. In the control group of 55 mice, receiving one-third

of a Dlm of toxin without spores, in one test two mice died and in two other tests one mouse died in each. The death of these mice is explained by the individual sensitivity of animals toward the botulinic toxin.

In two tests 60 mice of the second group were infected with 50 to 70 million spores and one-half Dlm of toxin. In these experiments, between 60 percent and 73 percent of the mice died from botulism. Among the 40 control mice, infected with spores alone, in one test three mice died. After the introduction of one-half Dlm of toxin into 40 mice in the course of one experiment, the death of 10 percent of the mice was observed; in the second test - 20 percent, which is also explained by the individual sensitivity of the mice to the botulinic toxin.

In five tests with 172 mice infected through the mouth with spores and toxin, 116 mice (67.4 percent) died from botulism. In the control group of 88 mice, infected through the mouth with spores, six (6.8 percent) died; there were 95 mice into which only toxin was introduced, and ten (10.5 percent) of these died from botulism. Cultures were made from the spleen and liver of the dead mice for the purpose of discovering the botulism bacillus. The identification of the cultures with this microbe was made by means of bacterioscopy and with the aid of a neutralization reaction.

The second series of tests was conducted on guinea pigs. As a precautionary measure, a lethal dose of dry toxin was titrated for the guinea pigs at the time that it was being introduced through their mouths. It appeared to be the equivalent of 0.0012

gramme. Just as was done in the first series of tests, the guinea pigs were given through the mouth simultaneously 75-100 million spores and one-third Dlm of toxin. A total of three tests were conducted. There were 33 guinea pigs infected by spores together with toxin, and of these 24 (72.7 percent) died from botulism. The control animals numbered 17, and they were infected only with the spores; three (18 percent) of them died. Among the 17 guinea pigs which were given only the toxin, two (11.8 percent) died. The death of these animals from one-third Dlm of botulinic toxin was connected, as has been described in the foregoing about the tests on mice, with the individual sensitivity which oscillates very strongly in guinea pigs. The results of the tests on mice and guinea pigs are presented on Table 9.

In these two series of tests on mice and guinea pigs, with a determined constancy, the infectious properties of the botulism bacillus were brought to light. The microbe, falling into the organism together with the toxin, apparently multiplies more rapidly and produced toxin in such a quantity that is completely sufficient for the animal's death. The toxin enters into the organism together with the spores and apparently shows a paralyzing effect upon the pharocytosis and penetrability of tissues on the part of the spores. is in turn conditions and permits the microbe to penetrate more quickly into the organs and to multiply.

On the basis of these experiments, it is possible to make the hypothesis that the toxin, introduced together with the spores, causes the sensibilization of the mice and guinea pigs toward such

doses of the toxin which produce the microbe that is penetrating into the organs. As a result, it appears completely sufficient for small doses of it to cause the death of animals from botulism.

The great majority of animals used by us in the tests died within a period of three to fifteen days after the infection. Usually at the beginning, a substantial loss of weight was observed. In some, a visible case of botulism developed. The animals remained under observation during 20 days after their contamination.

The data obtained, supporting the infectious properties of the botulism microbe, provided an incentive for us to analyze the course that botulism takes in the human being. The basis of this analysis was provided by the length of illness during various epidemics of botulism. If the botulism bacillus is possessed of infectious properties, then the sickness should be more serious and longer. As a result the human being develops an immunity and health is recovered, or the organism of the patient can not resist the infection and died.

We studied the works of a number of authors which were devoted to the observation of 21 botulism epidemics including 215 patients. Sixty-five of these died, 42 were ill for two weeks, 75 were sick one month, 26 remained patients for two months, and eight were ill for three months. In such a way, during the epidemics a slow reconvalescence was observed. This attests to the toxic-infectious character of the desease. Clinic workers observed in 1933 a large epidemic of botulism at Dnepropetrovsk and conducted very careful supervision over the patients. They also came to the consludion

Table 9. The Infectious Properties of Bac. Botulinus when Infecting Mice by Mouth

Number of test	Experiment					Control with spores				Control with toxin			
	Amount of toxin introduced	Number of spores introduced (in millions)	Number of living	Died from botulism	Percentage	Number of spores introduced (in millions)	Number of living	Died from botulism	Percentage	Amount of toxin introduced	Number of living	Died from botulism	Percentage
1	One-third of lethal dose for mice	25-35	16	11	68.7	-	-	-	-	One-third of lethal dose for mice	10	1	10.0
2		25-35	50	26	52.0	25-35	25	1	4.0		25	2	8.0
3		50-70	46	39	84.7	50-70	23	2	8.0		20	1	5.0
4	One-half of lethal dose for mice	50-70	30	18	60.0	50-70	20	3	15.0	One-half of lethal dose for mice	20	4	20.0
5		50-70	30	22	73.3	50-70	20	0	0		20	2	10.0
	Totals ...		172	116	67.4		88	6	6.8		95	10	10.5

Infectious Properties of Bac. Botulinus when Infecting Guinea Pigs by Mouth

1	One-third of lethal dose for pigs	75-100	13	11	84.6	75-100	7	2	28.0	One-third of lethal dose for pigs	7	1	14.0
2		75-100	10	7	70.0	75-100	5	1	20.0		5	1	20.0
3		75-100	10	6	60.0	75-100	5	0	0		5	0	0
	Totals ...		33	24	72.7		17	3	18.0		17	2	11.8

that botulism manifests itself as toxic-infectious. The slow process of reconvalescence in botulism is connected not only with the effects upon the organism of the toxin already present in the food but also with the poisonous activity of the toxin that is produced by the microbes which have penetrated into the organs and tissues.

The goal of our further research became the explanation of the transformations in the organism of animals, changes that are caused by infection with spores.

In order to explain the sensitivity of grey rats toward botulinic toxin, we set up corresponding experiments. It appeared

CHAPTER IV

THE PATHOGENESIS OF BOTULINIC INTOXICATION

Almost all domestic animals were shown to be sensitive to the botulinic toxin. Of the laboratory animals, most sensitive to subcutaneous introduction of toxin appeared to be guinea pigs and rabbits. Mice were somewhat more stable but also completely adaptable for experimental work. Animals are more sensitive to subcutaneous introduction of toxin than they are to its introduction through the mouth. According to Lippman (1910), a lethal dose of toxin for mice taken by mouth is 1,600 times larger than a subcutaneous one. The research of Bronfenbrenner and Schlesinger (1921) showed that the minimum lethal dose per mouth for guinea pigs is 1,000 times larger than one given subcutaneously and inside the peritoneum.

Gunnison and Meier (1930) occupied themselves with a study of the relationships between lethal doses of toxin by subcutaneous introduction and per mouth. They established that this depends on the appearance of the animal and the type of toxin. Burke, Elder, and Pishel (1921) in their research came to the conclusion that the absorption of the toxin from the intestines is influenced by various factors, in connection with which it is absolutely impossible to establish its lethal dose per mouth for animals.

In order to explain the sensitivity of grey rats toward botulinic toxin, we set up corresponding experiments. It appeared that rats die after the introduction under the skin of a somewhat larger dose of toxin than the one that kills a medium-sized rabbit.

If we take into consideration in this connection the great difference in weight between the rat and the rabbit, the great degree of stability of rats toward botulinic toxin becomes clear.

During recent years, two works have been published concerning the sensitivity toward the botulinic toxin on the part of other wild rodents. Himmelfarb (1936) established the existence of considerable sensitivity toward that toxin on the part of the spotted marmot, whereas Beridze (1936) did the same for the hamster who was shown to be more sensitive than white mice. The authors recommend the utilization of these animals for experiments in the study of botulism.

After the discovery of the botulism stimulant, one of the more important events in the study of this desease was the obtaining of experimental botulism in different laboratory animals. This provided the possibility of precipitating almost all symptoms of botulism in animals and of beginning the study of their pathogenesis. However, despite the almost fifty-year period of probing the pathogenesis of botulinic intoxication, this problem still remains in considerable degree unexplained.

In studying botulinic intoxication, great attention was paid to research on the penètrability of the stomach-intestinal tract for the botulinic toxin. Thus, it was established that the toxin in the alimentary tract undergoes insignificant destruction. Bronfenbrenner and Knox (1923) as well as Schlessinger proved that neither pepsin nor tripsin digest the toxin which is able to sustain the acidity of the stomach at 37 degrees during twenty-four hours.

The botulinic toxin was less stable in alkali surroundings, where its activeness dropped considerably over a period of twenty four hours. As a result of the instability shown by the toxin in alkali surroundings, it is more quickly eliminated from the intestines than from the stomach. On this basis, Bronfenbrenner made the hypothesis that its absorption transpires mainly in the stomach and in the upper part of the thin intestine.

Kagan and Matyqsh (1939), studying the influence of proteases upon the toxin of the botulism microbe, obtained somewhat different results. In their experiments, the toxin that underwent the activity of the stomach juice for 18-20 hours was completely inactivated. If the toxin remained in a 10 percent solution of pepsin or tripsin during four to six hours, its activeness did not change.

Dek (1926) showed in his tests that the toxin does not undergo disintegration when mixed with particles of tissue from the stomach and particles of muscle from the stomach wall of guinea pigs and mice over a period of one hour at 85 degrees temperature. After direct introduction into the stomach of guinea pigs and rats, the toxin was discovered in active condition during three to twelve hours.

In certain animals the walls of the stomach and intestines apparently are penetrable to only a small degree by the botulinic toxin, and for this reason animals can carry large doses of this poison. Thus, for example, Dek and Dzhibbard (1926) established the existence of a very high degree of stability in pigs for botulinic toxin when introducing it per mouth: after feeding it nine to ten million mouse doses, and animal weighing 6.3 kilogrammes remained completely healthy; not even a trace of toxin was discovered in

its blood. The toxin was introduced, in the case of two pigs, into a bandaged loop of the thin intestine. Then the blood from the vessels, passing through this fragment, was observed. In one instance, a negative result was obtained (the toxin did not pass into the blood); in the other, it was slightly positive. A similar test was undertaken on eight rabbits. In five of these, during two to two-and-one-half hours, a small quantity of toxin was discovered in the blood. The wall of the thin intestine in a dog also appeared to be impenetrable, according to the data collected by Hegram and Duke (1938), for the botulinic poison.

In 1942 Dek and Hoskins continued their experiments in the study of the penetrability by the botulinic toxin through the intestinal walls of monkeys. In the wall of the colon in these animals, a fistula was made, through which toxin was introduced. The monkeys that received this dose of poison through the mouth died from botulism. Of the four monkeys that were given the toxin in the same quantity straight into the nodule of the colon, only one died; three remained alive without showing any symptoms of the desease. The same dose of the toxin, killing the monkeys when introduced per mouth, when introduced into the colon appeared to be non-poisonous for the majority of the animals.

It follows that the botulinic toxin was not absorbed at all by the colon. This contradicts the hypothesis about the greater penetrability of the botulinic poison through the walls of the stomach and the upper part of the thin intestine in comparison with the remaining part of the intestinal tract.

Kushnir (1941) studied the influence of N-ion concentrations upon the activeness of the botulinic toxin. She established that the toxin, introduced into guinea pigs through the mouth, was possessed of its greatest degree of activeness at pH = 6.6 - 6.7. Lowering and increasing the concentration of H ions greatly lessened its activeness.

The activity of the poison is also reflected upon by the reaction of the stomach content, possible due to the absorbing properties of certain food products. Therefore, in order to have the desease develop not only a sufficient quantity of toxin is necessary but also favorable conditions for its absorption through the wall of the stomach and the thin intestine.

From the alimentary tract, the toxin penetrates into the blood. Its presence in the blood of patients ill with botulism and in the blood of experimental animals was proven by many authors (Friedman and Lorber, 1937, and others).

Clinical observations have received full corroboration in experimental research. According to the data of a number of authors, when the botulinic toxin is introduced per mouth into guinea pigs, rabbits and monkeys, it is discovered in the blood; the introduction of 0.1 milliliter of this blood into mice caused their death after visible symptoms of botulinic intoxication.

The conducted experiments speak of considerable stability on the part of the toxin, present in the blood. This also supports the work of Zakharina (1937) who introduced intravenously various doses of the toxin into rabbits and observed its circulation in

the blood during 24-48 hours. A minute study of this problem was conducted by us together with Bulatova (1948), and the results of this research are given in the following.

Thus the presence of botulinic toxin in the blood in the course of a certain time, which is at times of considerable length, as is the case in human beings ill with botulism, also in animals can be considered as proven.

The toxin penetrates from the blood into the organs, where it paralyzes the cells of different tissues. Naturally, by circulating in the blood, the toxin shows its effect also on the heart-vessel system. Clinical observations over the past twenty years provided considerable factual material on the paralysis of the heart-vessel system by the poison from botulism.

Clinical workers have observed in patients ill with botulism paleness of the skin, dryness of mucin, a good fullness of the pulse while the work of the heart has been weakened. Assuming that all of these symptoms depend upon the reduction of vessels through the action of the botulinic toxin, this hypothesis was corroborated in the experiments of Petrovski, Naumenko and Baturenko (1937) as well as Serebryana and Shkaver (1937). They all noted the reduction of vessels through the activity of the botulinic toxin as it affected the vessels in the ear of a rabbit.

The vessels of the kidneys and lungs, according to the data of Petrovski, Naumenko and Baturenko, are affected by the toxin in two phases. At first this precipitates an enlargement and later a contraction. The authors consider the botulinic toxin to be a

strong vessel poison. In studying the condition of the capillaries in botulism patients, Steinberg (1937) also discovered the contraction of the arterial node in the capillaries. Certain authors (Mirtovski and Govseev, 1937) explain many of the symptoms attending botulism by the collapsing condition of the vessels at the periphery. Usually, the patients complain of poor sight ("sight like through a cloud," "a curtain has dropped before the eyes,""it is dark in front of the eyes"), spinning of the head, head-aches. All of these symptoms can be, according to the authors, connected with the collapse in the condition of the vessels in the main part of the brain. Dixon (1915) considers that changes in the nerve cells appear to be the result of lessening the access of blood to them. This problem requires further study.

Dixon (1915), Semerau and Noak (1919), Wilbur and Ophules (1924), Wartin (1932) announced that botulinic toxin causes the cells of vessels to become damaged, especially the endotheliums of the cappilaries, expressed in injury to their wholeness. It is necessary to mention that the mentioned authors were studying the pathomorphological transformations on the basis of very small material (Dixon and Wartin on four cases, the remaining authors on one). For this reason, their data can not be of serious importance in the study of this problem.

Circumstantial observation of damage to the vessels in botulism was conducted on considerable material by Kuraev (1937). The author mad a pathologic-anatomical study of 23 botulism cases, in the course of which he observed all tissues and organs. As a result

there was established a certain common type of paralysis for various organs, among which was the vessel system. Kuraev notes that the changes most often met with in the vessels system are "swelling of the endotheliums, clearly expressed in the endothelium of the cappilaries in fine vessels; further it is transformed into the processes of necrobiosis. The process of swelling of cells and necrobiosis in the medium and small vessels is noticably spread out in the middle membrane."

On the basis of this data, it is clear that during a paralysis by the toxin of endothelium cells, the vessels are substantially injured in the impenetrability of the vessel walls. The botulinic toxin penetrates from the blood-carrying vessels into the surrounding tissue and precipitates in it damage to the cells.

The path by which botulinic toxin reaches the central nervous system for the present time has not been definitively established. On this question, the literature available contains a small amount of contradictory observation. The clinical research on botulism provides a considerable number of facts, indicating the selective paralysis of the central nervous system. The presence of a special sensitivity of the nerve tissue toward the botulinic toxin was established by the experiments of Kempner and Shepilevski. These authors expressed the hypothesis on the transfer of the botulinic toxin with the flow of the lymph or blood and possibly the spreading of it through the neurons to the nerve cells. Lazaris, Minervin and Friedman (1937) also came to the conclusion that the nervous system possesses a substantially expressed sensitivity to the botulinic toxin.

Contradictory results were obtained in the experiments of Burke, Elder and Pishel (1921). Kolimen (1924), repeating the tests of Kempner and Shepilevski, also obtained negative results. He explains the discovery of toxin in the brain tissue, when it was introduced parenterally on experimental animals, through its presence in the cappilaries.

This explanation does not agree with the findings of Shapiro and Nikolenko (1937). Examining the presence of toxin in the organs of people who had died from botulism, they discovered it almost exclusively in the spinal cord, at the same time that it was absent from other tissues. It is characteristic that toxin was found in 100 percent of the cases where persons above 16 years of age had died from botulism. Finally, the problem of the special sensitivity on the part of tissues in the central nervous system toward botulinic toxin still remains unsolved and requires more study.

The question of penetration by the toxin into the central nervous system was studied by Mirtovski and Govseev (1937). Basing their work on a large amount of clinical data, they reject the hypothesis of Burke, Elder, Pishel and Kolimen on the possibility of penetration by the toxin along lymphatic paths and the axial cylinders of the nerve fibres; they support the overwhelming importance of the hematogenous channel for penetration by the botulinic toxin into the central nervous system.

As corroboration of their point of view, they call attention to the research by Rappoport and Lifshits (1937) who could not discover toxin in the liquiform of 17 botulism patients. The changes

of a pathological nature in the liquiform also appeared to be completely insignificant: only a small enlargement of the globulines was discovered. Insignificant transformations in the liquiform point toward the absence of a meningeal reaction. For this reason, the hypothesis about the distribution of the toxin along the parineural area is of little credence. In the opinion of Mirtovski and Govseev, the toxin penetrates into the nervous system through the heavily damaged walls of the brain cappilaries.

This point of view agrees with the experiments of Silberman (1938) who studied the poisonous effects of botulinic toxin when introduced into rabbits by various methods. During the intravenous, subdural and subarachnoidal introduction of the toxin, the rabbits died simultaneously; the size of the lethal dose and the clinical picture were also identical. Last of all the rabbits died, when toxin was introduced subcutaneously. Sulfur therapy for botulism in animals, when the serum had been introduced subdurally and intravenously, provided equivalent results; the subcutaneous method of introducing the serum appeared to be less effective.

Observations corroborate the opinion of Mitrovski and Goseev on the hematogenous channel along which the toxin penetrates into the central nervous system as a result of damage to the endotheliums of the brain vessels. The point of view expressed by these authors seems to be the most probable, although it requires more thorough experimental study.

In a second work by Zilber and Govseev, on the basis of their pathologic-anatomical research, they present the hypothesis that

the toxin penetrates into the central nervous system by hemato-liquiform-encephalic means.

A uniformity in opinion has yet to be achieved also on the question as to which part of the nervous system is stricken hardest of all in botulism. In the view of Mitrovski, Govseev, as well as Zilberman and others, the ganglionic cells of the central nervous system are paralyzed fundamentally in botulism. Other authors (Dixon and Shevki, 1923; Edmunds and others, 1923 and 1924; and also Koritski, 1937) explain all of the symptoms of botulism as the effect of the toxin (similarly to curare, South American arrow poison) upon the ends of the motor nerves of the voluntary musculature. The poisonous activities of the toxin precipitate a condition leading to rapid fatiguing of the nervous system. The ends of the sensory nervous system remain undamaged.

Kuraev on the basis of pathologic-anatomical research (1938) came to the conclusion that "the place of primary fixation for the toxin is served by the supporting tissue - uniting, reticulinous, elastic fibres, a chromatic substance - from which the activity of the toxin is distributed among all reticulin-endothelial systems, then on the parenchymatous elements of different organs (the kidney cells, flat and laterally striped muscles, the cells of the endothelial-vessel nervous systems), after which already on the background of these transformations the whole clinical symptom complex is played."

It can be seen from the survery of literature that has been brought out that the pathogenesis of the botulinic intoxication remains unexplained. Only separate stages in this process have been established.

Certain scientists attempted to approach an explanation of the problem by means of studying the impact of the toxin upon the isolated organs. Three works have already been conducted in this direction. One of these was done by Petrovski, Naumenko and Baturenko (1937) who studied the vessel-contracting effect of the botulism toxin and also established its ability to stimulate the flat musculature of the intestines. In the second project Shkavera (1941) and Serebryanin (1937) also note the ability of the botulinic toxin to strengthen the contraction of the intestines. The authors state the hypothesis on the selective activity of the botulinic toxin directed toward the parasympathetic nervous system. However, this research did not provide any definitive solution to the question as to the mechanism of the botulinic intoxication.

1. Reaction of Cells in Vessels of Animals and Man to Botulism Toxin

As can be seen from the data in the literature mentioned in the foregoing, the study of botulinic intoxication over the past 15 years brought about the explanation of many hitherto unknown aspects of this process. A series of facts have been established, showing that botulism not only strikes at the nervous system but also at the cardiac-vascular and muscular systems.

These conclusions of clinical workers (Shteinberg, Katsnelson, Papernyi, Kutsygin, Kheifets, Changli-Chaikin, Abramova, 1937), in part corroborated through pathological-anatomical research (Kuraev, 1937), still require experimental checking. Apart from this, careful observation of separate botulism epidemics showed that the course of many cases could not be explained in full only

on the basis of an exogenous intoxication during which, as is known, the infectious role of the botulism microbe is rejected.

In a botulinic poisoning of a human being or animals, the botulism microbe enters the organism together with the toxin. For this reason, when explaining the mechanism of the botulinic intoxication, it is necessary to take into account the part played by these two factors.

The above cited experimental research showed that small (relatively speaking) quantities of spores from the botulism bacillus, introduced through various channels into the organism of animals, can remain in the organs for a long time without losing their viability. In connection with this, it is important to explain the influence manifested upon the cells by the spores from the botulism microbe that is found in the tissues. Does this process appear to be one of carrying without reference to the desease, or is it a genuine infection precipitating corresponding changes in the cells and the fluids of the organism?

A solution to this problem is of very great theoretical and practical significance. It is a prerequisite for a correct understanding of the pathogenesis of botulinic intoxications as well as for planning practical measures in the struggle against the botulism infection.

As was shown in the foregoing, quite often products that are contaminated by the spores from the botulism microbe are used in food after boiling. However, as was shown by the experiments of Komkov (1935) even a lengthy boiling of a product does not kill

the spores of this microbe. The effect of these spores upon the organism of the human being and animals is unknown.

The mechanics of botulinic intoxication with ready toxin in man and animal also remain not completely clear. As the research of several authors has shown, the toxin is absorbed mainly in the upper part of the alimentary tract, in the stomach, and in part in the thin intestine. From these organs, the toxin passes on into the blood where it can circulate for a considerable time. In certain cases of botulism, it appears in the blood and then disappears and then appears again. A substantial quantity of the toxin, apparently is secreted with the urine (Fridman and Lorber, 1937). When entering into the blood carrying vessels, the toxin causes their paralysis. According to the data of Shteinberg, Kutsygin, and Kheifets (1937), a strong contraction of all blood vessels also takes place under the influence of the toxin.

In order to check on the correctness of these clinical results, we conducted the experimental research described in the following.

In the pharmacological and physiological research on the reaction of vessels, the method of isolating organs as instituted by Kravkov and Pisemski in 1912 is widely applied. At the present time, it is used mainly for the study of the effect by various substances upon the vessels of animals and man.

In the literature on botulism, there are three works of Soviet scientists devoted to the study of the reaction by vessels in the ears of rabbits and the feet of frogs to botulinic toxin.

Petrovski, Naumenko and Baturenko (1937) in their experiments were able to obtain a sharp contraction in the vessels of rabbits' ears when passing a solution of active toxin through them and a much weaker contraction with the boiled toxin.

Serebryanaya and Shkavera (1937) studied this problem and obtained contradictory results. In their tests on the vessels in the ear of a rabbit, a toxin inactivated through boiling gave a sharper contraction than a toxin which had not undergone boiling. The authors explain that the effect of the amines in the surroundings, for which the botulinic toxin was prepared, is the controling factor. The contradictory results from these observations were apparently caused by the differing composition of the surroundings for which the toxin was prepared. The experiments were conducted with a fluid botulinic toxin, because of which the effect upon the vessels of different substances in the form of admixtures was especially strong.

In our study of the effect had by the botulinic toxin upon the vessels of animals and man,we also utilized the Kravkova-Pisemski method but took for this purpose a toxin that had been cleaned of all admixtures. The utilization of such a toxin for our tests, according to our hypothesis, should have given more accurate results reflecting the reaction of the vessels to the poison.

The method of Kravkova was at first applied to the work on isolated ears of rabbits, then it was used for work on the rear half of the body of rats and guinea pigs, human kidneys and other things (Pisemski, 1912; Zakusov, 1904; Waldman, 1940). In experiments on

bacterial toxins, this methodology was widely used by Kravchenko and Galanova (1941) and Zamuri (1936) in their research on the immunological condition of cells in vessels during various infections and immunizations.

The work with isolated organs according to the Kravkova-Pisemski method does not present great difficulties. It requires only a strict adherence to the methodology in each test and, thus, identical results are obtained. The main aspects, which it is necessary to take into consideration, are the maintenance of constancy in the temperature of the fluid dripping into the organ and the pressure of the liquid column.

After isolating the organs, the vessels were washed from blood with the help of a large syringe. In order to do this, the tip was placed in the artery and a Ringer-Lokk solution heated to 37 degrees was passed through until a transparent fluid was attained. Then the organ was fastened on a glass plate with corks and pins. Finally the heated Ringer-Lokk solution was passed through the organ with a pressure column of 48 centimeters until a constant number of drops per minute had been established (see the schemat of this apparatus on Figure 2).

This type of washing in each test took place for not less than 10 to 30 minutes, depending upon the reaction of the vessels to the fluid passing through them. The quantity of drops of the fluid was regulated by means of changes in the width of the strip of filter paper along which it dripped. As a convenience in computing, during the work on the ears of rabbits and the rear half

Figure 2. Construction of the Kravkova-Pisemski Apparatus. 1 - shelf for containers with solution; 2 - containers with Ringer Lokk solution and toxin; 3 - rubber tubes through which the Ringer Lokk solutions and toxin drip toward the coils; 4 - coils, serving to heat the Ringer-Lokk solution and the cultured toxin; 5 - vessel with water, where the coils are located; 6 - electrical temperature regulator with heater for heating water in the vessel with coils; 7 - thermometer, showing temperature in the vessel with coils; 8 - thermometer, controlling the temperature of the fluid entering the isolated organ fastened on to the Bunsen stative; 10 - the isolated organ; 11 - vessel for fluid dripping from the organ.

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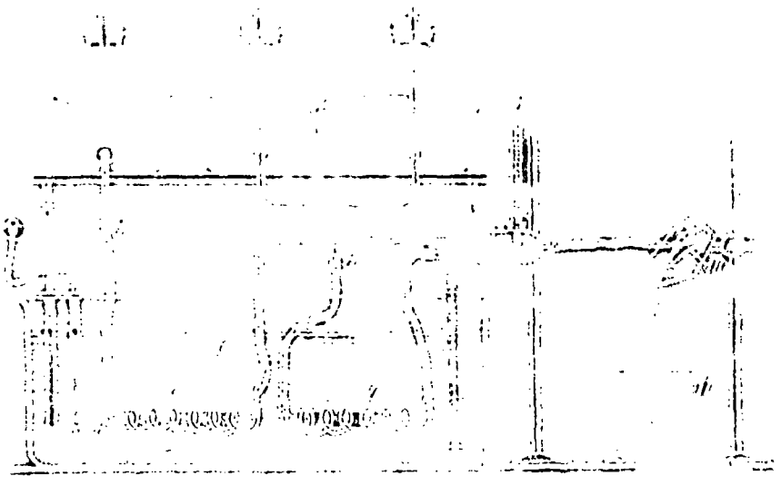
of the body of guinea pigs, the number of dripping drops at the beginning of the test these were established at no less than 80 and no more than 150 per minute. In the experiments on the human kidney, the quantity of fluid dripping in one minute was measured in a



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graduated cylinder.

After a constant amount of fluid had been established for the drippings within one minute, a tabulation was made for the last three minutes. The interval between computations amounted to one minute. The number of drops or cubic centimeters of fluid, dripping during three minutes, comprised the calculated arithmetic mean. Therefore, in the course of five minutes a solution of toxin from the botulism bacillus was sent through the vessels. After this, again during three minutes with a preceding interval the number of drops of fluid dripping in one minute was computed. The results consisted of one-half the arithmetic mean of the figure. After the toxin, the Ringer-Lokk solution was released until the establishment of a constant number of drippings per minute. The process of attaining the preceding quantity of dripping fluid continued for no less than 10 to 15 minutes; before this time, a constant reaction of the vessels could not be ascertained.

The quantity of Ringer-Lokk solution, after release of the toxin, usually was always somewhat smaller than at the beginning of the experiment.

If the amount of dripping fluid after the toxin has been released increases, then such a test is excluded since it was begun when the reaction of the vessels to the Ringer-Lokk solution was not yet constant.

In setting up the tests, a dry toxin was prepared from Bac. botulinus type A or B by means of extracting it from a solution of ammonium sulfate. The toxin floated to the surface and was collected

into a cup and carefully wrung out to free it from the fluid. Then the toxin was dried, mashed against a mortar into a fine powder, and titrated on mice to calculate its activeness.

In these experiments, the toxin used had considerable power. A lethal dose for mice comprised 0.0000[illegible] to 0.0000001 grammes. Dry toxin was watered down 100 times and then underwent analysis in a coloidal sack, at first 16 to 18 hours in running tap water, and then for twenty-four hours in distilled water.

By such a method, it was possible to obtain toxin free from many admixtures present in the fluid toxin. It did not include amines, since these do not settle in ammonium sulfate. Apart from this, it was free from certain albumins in the surroundings which did not fall into the sediment in the concentrated solution of ammonium sulfate. With the aid of dialysis, it was possible to clean it from the admixture of various salts and possibly also of many other substances.

After the dialysis, the poisonous properties of the toxin solution decreased two to three times. This was dependant apparently upon the fragmentary elimination of certain dispersion on the part of the toxin at the time of the dialysis. In the tests, therefore, solutions with a large quantity of toxin were used.

In order to observe the tonicity of the vessels when releasing the solution of botulinic toxin through them, we used the vessels from rabbits' ears, the rear half of the body from guinea pigs, and the vessels of the human kidney. Vessels from isolated organs appear to be very favorable subject matter for research,

since they preserve their viability for a long time.

Kratkov (1923) concerned himself over many years with the study of the reaction of vessels to various substances. He came to the conclusion that "the ears of rabbits, preserved without any special precautions; the ears of hares, killed during hunting; the ears of calves from butcher shops - all continue to react slightly to vascular substances and other actions for several days, and when preserved on ice for several weeks. Under special conditions of conservation, the vessels of the ear can manifest their viability over an uncalculated long time."

In the first series, we established tests with the vessels from the ears of 15 rabbits. As a precautionary measure in the rabbit the ear artery was separated, bound with two ligatures, and then between the ligatures the ear was cut with a sharp razor. Then the ear vessels were washed with a heated Ringer-Lokk solution. From this moment, according to the constant tonicity established which was calculated on the basis of fluid dripping out in one minute, we commenced to release the toxin at first in a strong and then in a much weaker solution. The first solution of toxin was 1:50,000; the second was 1:25,000; and the third was 1:10,000.

For six rabbits in this series, it was customary to allow a solution of the toxin to enter through one ear and a solution of inactivated toxin through the other ear. In order to inactivate the solution 100 times, it was boiled for 15 to 20 minutes in a water bath. The vessels of the ears from all 15 rabbits reacted by contracting to the botulinic toxin (Table 10).

Table 10. Reaction of Vessels to Botulinic Toxin

Number of series	Number of experiments	Experiment or Control	Average contraction of vessels (in percent)		
			toxin 1:50,000	toxin 1:25,000	toxin 1:10,000
		Reaction of vessels in ears of rabbits			
1	15	Experiment	23.2	36.5	36.9
	6	Control with boiled toxin ...	4.0	6.0	6.5
		Reaction of vessels in rear part of body in guinea pigs			
2	10	Experiment	23.1	25.2	35.8
	5	Control with boiled toxin ...	4.6	6.4	3.8
		Reaction of vessels in kidney of human being			
3	10	Experiment	22.1	25.5	32.7
	3	Control with boiled toxin ...	4.9	7.4	16.7

In the first distribution of the unboiled toxin, the vessels underwent an average contraction of 23.2 percent; in the second, 36.5 percent; in the third, 36.9 percent. Thus, in the second and third distributions of the toxin the vessels in the ears of the rabbits reacted almost identically in their contraction.

The vessels in the ears, through which the inactivated toxin was passed, gave an insignificant contraction: in the first distribution

of the boiled toxin the average contraction was four percent, and in the second and third between six percent and 6.5 percent.

In these experiments, completely accurate results were obtained which portrayed the reaction of the vessels in the ears of rabbits to the botulinic toxin. The higher the concentration of toxin, the stronger was the contraction of the vessels. When the botulinic toxin was inactivated by boiling, cleansed by a precautionary precipitation and submitted under dialysis, it caused a very insignificant contraction in the vessels of the rabbits' ear - especially in comparison with natural toxin.

The second series of tests was made on 15 guinea pigs. The animals were killed by striking their heads against a hard object. Then the stomach nodule was opened, bandaging with ligature the stomach aorta, and cutting the animal's body with sharp scissors in half. In the aorta of the rear half of the guinea pig's body, a glass tube was inserted and the vessels washed with a syringe using the heated Ringer-Lokk solution. After this, the preparation was strengthened on a glass plate. Again the Ringer-Lokk solution was passed through uhtil the establishment of a constant quantity of fluid dripping during one minute.

In this series of experiments, the action of the same toxin distribution was observed as in the preceding one. The reaction of the vessels was observed toward toxin in 10 guinea pigs; the toxin was not boiled. In letting through the first and second distribution of toxin, the vessels of the animals showed an average contraction of 23.1 to 25.2 percent; and in the third, 35.8.

The toxin inactivated through boiling precipitated a contraction in the vessels of five guinea pigs to the extent of 4.6 to 6.4 percent (Table 10).

The data cited from the tests with the vessels of guinea pigs do not differ from the results of tests in the first series, which were conducted with vessels from the ears of rabbits. The vessels of guinea pigs reacted with a considerable contraction to the botulinic toxin and gave almost no reaction to the toxin that had been disintegrated through boiling.

In the third series of experiments, we studied the reaction of vessels from the human kidneys to the botulinic toxin.

Vessels from the organs of man after death also preserve their viability very long. According to the research of Shkavera (1923), the reaction of the kidney vessels begins to weaken only during the second or third 24-hour period after death. Waldman (1940) considers that the arteries in a cadaver preserve the possibility of a reaction in the course of 24 to 48 hours after death.

In order to obtain accurate results in tests on human vessels, it was very important to use the organs from human cadavers which had not lost their vessels from the desease. In the study of the reaction by vessels to botulinic toxin, we took kidneys from human cadavers that had died from street accidents. It was possible in most cases to obtain the organs within four to five hours after death. A total of 13 experiments was undertaken (see Table 10). The methodology used was the same as that applied to the previous experiments. A glass tube was inserted into the artery of the kidney, and a Ringer-Lokk solution was passed through

until there was no more blood in the fluid dripping out. After this, a solution of the botulinic toxin in the same series and in the same distribution as in the preceding tests was passed through the vessels of the kidney.

For the first culture of the toxin, the vessels of the human kidney showed an average contraction of 22.1 percent; in the second, 25.5 percent; and in the third, it was 32.7 percent. The contraction of the kidney vessels when treated with the boiled toxin in the first culture amounted to 4.9 percent, in the second 7.4 percent, and in the third 16.7 percent. The results achieved in the tests on human kidneys do not differ from the preceding.

Thus the vessels from rabbits' ears, the vessels from the rear half of guinea pigs, and the vessels from the human kidney provided identical reactions to the botulinic toxin. Contraction of the vessels in these organs differ only in their intensity. This can be explained through the mechanism of direct effect which the botulinic toxin has upon the flat musculature and upon the sympathetic endings of the vasoconstructors in the vessels of animals and human beings. In the pathologic-anatomical observations of Kuraev (1937, 1938) data are provided supporting this position.

A stronger contraction was provided by the vessels in the ears of rabbits and a weaker one in the vessels of the human kidney. The weakest contraction of vessels amounted on the average to 22 percent, whereas the strongest was no greater than 37 percent.

With the aid of the toxin that had been cleaned through sedimentation and dialysis, we were able to accurately reveal its effect upon the vessels of animals and man. The toxin which had

been disintegrated through boiling in a water basin precipitated a very weak reaction in the vessels of animals.

In our opinion, these experimental observations provided full corroboration for the observations of clinical workers on the condition of the cardiac-vascular system during botulism. In the clinic, patients are observed to have a full pulse although the work of the heart is weakened, and also a pale skin, as well as dryness of the mucous glands. Clinical workers who had studied this problem (Shteinberg, Katsnelson, Kutsygin and Kheifets, 1937) announced their hypothesis that these phenomena take place in connection with the vessel-contracting effect of the botulinic toxin. Our tests have corroborated this hypothesis.

In such a way, on the basis of clinical observations and experimental research, it can be stated definitely that the botulinic toxin appears to be a very strong vascular poison which causes contraction of the vessels.

2. Penetration of Toxin from the Botulism Microbe into the Central Nervous System

As has already been shown in the foregoing, the path of penetration by the toxin into the central nervous system remains unexplained to the present. Its presence in the blood during botulism poisoning in human beings as well as in experimental animals is no longer a matter of opinion. Being in the blood vessels, it causes their contraction. Apart from this, as was proven by Kurasv (1938) on the basis of considerable pathologic-anatomical material, the

botulinic toxin causes damage to the walls of vessels - mainly the precappilaries and the cappilaries, in all human organs. It seemed completely natural that the channel of forward movement by the toxin into the tissue of the organs, among which is the nervous system, should lie through the damaged wall of the blood vessels. However, such a path for its passing into the main part of the brain did not obtain even primary corroboration in experimental observations.

No uniformity of opinion was achieved as to which part of the nervous system is paralyzed by the botulinic toxin. Dixon and Shewke (1923), Koritski (1937) consider that the toxin of this microbe functions primarilly on the peripheral nervous system and disintegrates the endings of the motor nerves.

Visibly expressed symptoms of paralysis to the nervous system during botulism gave rise to the hypothesis on the special sensitivity of it to the botulinic toxin. This point of view was supported by the research of Lazaris, Minervin and Fridman (1937).

Certain authors reject the special sensitivity of the central nervous system toward toxin. Thus, Burke, Elder and Pishel (1921) assume that the toxin enters the central nervous system by means of the lymphatic paths and the axial cylinders of the nerve fibres.

Mirtovski and Govseev (1937), on the basis of clinical observation, agree only to the hematogenous path for penetration of the toxin into the tissues, including the nervous tissue. They reject the lymphogenous channel for penetration of the toxin into the nerve tissue on the foundation that meningeal reaction is

absent from the patients. In connection with the absence of the meningeal reaction, these authors consider that little credence should be placed in the forward movement of the toxin along the lymphogenous path and also in the perineural area. Besides, very insignificant transformations are observed in the spinal cord fluid during botulism. Support of this is also found in the absence of toxin from the liquiform of botulism patients when it is present in the blood, which was established by Rappoport and Lifshin (1937).

Frideman and Elkels (1934) reject the hematogenous path for the penetration of the toxin into the nerve tissue. On the basis of their research, they came to the conclusion on the complete inpenetrability of the cappilary walls in the central nervous system for the botulinic toxin. In their opinion, the toxin circulates in the vessels and does not reach the nervous system.

As we can see, many contradictory opinions are voiced on this question. It, therefore, seemed to us very important to explain which of these was closest to reality.

With this goal, a series of tests were undertaken (Matveev, Bulatova, 1948) which would lead to an explanation of the channel used by the botulinic toxin for its penetration into the central nervous system.

The first series of experiments was made on 22 rabbits. A large quantity of toxin was introduced intravenously into the rabbits, and it was measured by the quantity of Dlm for mice. Once the animals received from 10,000 to 15,000 doses lethal for mice. Part of them received subcutaneously 24 hours prior to the test one-half of a lethal dose of toxin for a rabbit, and on the

following day intravenously 2,000 to 10,000 Dlm for mice. This quantity of toxin was injected 24 hours before the experiment in order to cause a paralysis of the vascular system in the animals and create favorable conditions for the penetration of the toxin into the tissue. After the intravenous injection of large doses of toxin into the rabbits, they either dead within two hours or died in the majority during three - four - five hours or in separate instances during 10 to 24 hours.

For the purpose of explaining the channel of penetration by the toxin into the main part of the brain, experiments were made for its presence in liquiform, blood, and an extract from the main brain. Liquiform from rabbits was taken by means of a suboccipital puncture during one to two hours after the injection and in the period of their agony in the course of three - four - five hours, 10 to 15 minutes before death. After the death of the rabbits, the main part of the brain underwent a careful washing with a physiological solution in a Petri dish. The washing was repeated several times, until the tissue of the main brain was not completely cleansed of blood. From various parts of the brain, pieces were taken and comminuted on a slide to which double their volume of physiological solution was added; the suspension was kept for two hours on the table for extracting the toxin. Blood for the biological test was taken from the heart immediately after the death of the rabbit.

A biological experiment on mice was made with the suspension from the main part of the brain, liquiform and blood for the purpose

of discovering the botulinic toxin. The animals received subcutaneous injections in all tests of 0.1; 0.5; 1.0 milliliter of the suspension from the brain or blood and o.1; 0.5 milliliter of the liquiform.

In all experiments with the suspended matter from the main part of the brain and the liquiform, the biological test for toxin was negative at the same time that toxin was found in the blood of 19 rabbits in large quantities. The blood of certain rabbits (No.3, 7, 10, 13, 22 and 21), diluted four to five times with a physiological solution in quantities of 0.1; 0.5; 1 milliliter, caused the death of mice.

Interesting facts were established through these tests. It appeared that in the presence of a large quantity of botulinic toxin in the blood of animals, the toxin is not found in the spinal cord fluid or in the main part of the brain.

The negative results obtained in our experiments on the liquiform of rabbits correspond in full with the data of Rapoport and Lifshits (1937), obtained from their research on the liquiform of human beings ill with botulism. These authors found toxin present in the blood of 17 patients but did not discover it in their spinal cord fluid. It follows that the botulinic toxin does not penetrate into the liquiform of rabbits or human beings, even when it is present in large quantities in the blood.

This fact completely coincides with a second important aspect, the absence of the toxin in emulsions from the main part of the rabbits' brain. According to the theory about the hematoencephalic barrier, the latter impedes penetration into the brain

of many substanced from the blood, which have not first of all reached the spinal cord fluid. Any kind of substance can enter the brain only through the liquiform. In the given instance, the absence of the toxin from the brain suspension would seem to support this point of view.

Frideman and Elkels, on the basis of the absence of toxin from the main part of the brain, came to the conclusion about the absolute inpenetrability of vessel walls in the central nervous system for the botulinic toxin.

In view of the fact that in botulism the symptoms of paralysis in the central nervous system are expressed very clearly, this especially emphasizes the "affinity" of the nerve tissue for botulinic toxin. Therefore, the conclusions of individual authors about the special "affinity" of the nervous system toward botulinic toxin appear to be correct. The absence of toxin from the brain suspension, in our opinion, could be dependant upon the fact that the toxin extraordinarily stably was adsorbed by the tissue in the main part of the brain and extraction in the course of two hours was insufficient for separating it.

In order to check this hypothesis, we established supplementary experiments. Suspended matter from the brain of six rabbits that had received toxin was intraduced twice into mice and guinea pigs. The mice remained healthy, but the guinea pigs died from botulism. However, the guinea pigs that had received this suspension together with anti-botulinic serum remained well. Besides, the suspended matter from the main part of the brain of rabbits

together with the double volume of physiological solution were placed into a thermostat. After keeping this in a thermostat for two to seven 24-hour periods, the tissue from the main part of the brain began to fall apart and consequently freed the botulinic toxin. The toxin was discovered through the reaction of neutralization with anti-botulinic serum in nine of the 16 tests.

It is important to mention that while the brain remained in the thermostat, the toxin appeared unevenly in the extract: in some experiments more rapidly, in others slower, and in certain ones it did not appear at all. The botulinic toxin is possessed of considerable stabiäity to the action of temperature and products disintegrating the albumin. However in separate instances, apparently under the influence of disintegrating products from the brain tissue, it undergoes inactivization.

The described experiments convincingly show the considerable "affinity" of the tissue in the main part of the brain for the botulinic toxin. The former can adsorb the latter in a very stable way. Only during disintegration of the nerve tissue in individual cases is the toxin liberated. Disintegration of the tissue in the brain of rabbits within the organism of guinea pigs took place with a freeing of the toxin, and the guinea pigs died from botulism.

These tests also establish that toxin can penetrate into the main part of the brain eluding the liquiform, since the toxin was absent from the latter in all experiments, although it was present in the blood in a large concentration.

The forward movement of the toxin into the main brain of rabbits along the axial cylinders of the nerve fibres is excluded. In order to reach the main brain by this path, providing that this

is at all possible, the toxin would need a longer period of time than that observed in our experiments.

The second series of tests was made on mice and guinea pigs in order to check the experiments of Frideman and Elkels (1934).

We set up five tests in the course of which 20,000 Dlm of the botulinic toxin was injected intravenously into each mouse. The animals died within one and one-half hours from botulism. This dose of toxin was selected for the purpose of creating a large concentration of it in the blood, which should have provided the conditions for its penetration into the brain through the walls of the capillaries. In mice that died from botulism, the main brain was extracted and washed several times in a physiological solution. A suspension was prepared from the brain by means of carefully comminuting it in a mortar and adding a double volume of physiological solution. The suspensive matter was injected subcutaneously in quantities of 0.5 milliliter each into 10 mice and two milliliters each into four guinea pigs. All of the mice remained alive, but the guinea pigs died from botulism. In the brain tissue, we found toxin which had been stably adsorbed and thus lost its poisonous properties. Therefore, the mice stayed alive.

In the following six experiments the mice received intravenous injections of 4,000 Dlm each. The animals died from botulism within two hours. During a one-time introduction of the brain suspension from the dead mice at 0.5 milliliter into 12 mice and at two milliliters into six guinea pigs, the mice remained healthy but

the guinea pigs became ill with botulism. After a second introduction of the brain suspension from mice, all guinea pigs died on the third or fourth day from botulism.

In the course of 32 experiments, a total of 1,000 Dlm was injected into each mouse; the animals died within three hours. The suspensive matter from their brain was introduced once into 24 mice in the quantity of one milliliter and twice into 12 guinea pigs with two to three milliliters each time. Of the 12 guinea pigs, seven died from botulism; all mice remained healthy, without any visible desease.

In order to explain the stability of the mice to the toxin, after twice injecting the brain suspension from mice and receiving intravenously 4,000 Dlm of toxin each, we set up nine supplementary experiments. In the latter, the brain suspension was introduced twice into 18 mice with 0.5 milliliter and into nine guinea pigs with two milliliters each. All mice and guinea pigs died from botulism, with the exception of the guinea pigs that had received two milliliters of anti-botulinic serum.

The conducted tests clearly show the presence of the botulinic toxin in the brain tissue of mice receiving it intravenously. It is important to note that during a single injection into mice of the brain suspension from mice, which contains the adsorbed toxin, these animals did not manifest the botulism desease at the same time that guinea pigs receiving the same suspension died from botulism.

The lethal dose of toxin, injected intravenously, was ten times larger for guinea pigs than it was for mice. In all tests the guinea pigs received the brain suspension only two to four

times in larger quantities than did the mice. Probably the disintegration of the brain tissue of the mice and rabbits in the organism of the guinea pigs took place together with the liberation of the adsorbed toxin, and they died from botulism.

The completed observations show that the experiments of Frideman and Elkels were erronious and their conclusions on the absence of the botulinic toxin from the main part of the brain in mice incorrect.

In summarizing the achievement of our observations, we see that in the tests on rabbits and mice with intravenous injections of the botulinic toxin identical results were obtained. In these animals the toxin penetrated into the central system where in considerable quantity it was adsorbed by the nerve tissue, temporarily losing its poisonous qualities. When introducing the emulsion from the brain of mice in quantities of 0.5 to 1 milliliter, the animals remained well. However if the brain tissue containing the toxin underwent disintegration in the thermostat prior to injection into the mice of if it was injected into guinea pigs, the toxin liberated itself and the animals died of botulism.

All of this data prove that the central nervous system in rabbits is accessible to the botulinic toxin which penetrates through the vessel walls in the brain. Corroboration for this appears to lie in the absence of it in the liquiform of these animals. In the case of mice, penetration of the toxin into the main part of the brain takes place apparently through this channel.

On the basis of all of our data, it is possible to make only one conclusion: the botulinic toxin penetrates into the central

nervous system of rabbits and mice through the walls of the vessels in the nerve tissue.

Apparently this path for the penetration of the botulinic toxin into the main brain in sick human beings is also the principal one. This hypothesis is supported by the fact that in the presence of the toxin in the central nervous system inside of people who have died from botulism it is at the same time absent from the liquiform.

3. Reaction of Vessels in Experimental Animals during Symptomless Infection

The botulinic intoxication, causing acute illness, is accompanied by a strong paralysis of the vascular system. The question arises: how does a long-lasting symptomless botulinic infection reflect upon the vascular system of animals? The possibility of creating such an infection in experimental animals was established by us in the preceding experiments.

Up until recent times, nobody among researchers attempted to explain what happens in the organism of the experimental animal when it is infected with a small quantity of spores that do not precipitate the death of the animal.

The study of this problem is a prerequisite for a correct presentation of the botulinic intoxication. Observation on the condition of the vessels at the moment of discovering the botulism stimulant in the organs of animals, in our opinion, will help to explain what transformations occur in the organism at this time and whether the spores cause a real infection or whether this is only

the inculcation of other bodies as is claimed by certain authors.

Making use of the writings of Waldman "that in infections and intoxications, acute as well as chronic, we are faced with a paralysis not only of the vaso-motor centers but of the vessels themselves, their tonicity," we launched upon a study of this problem from the immunological point of view.

In a chronic intoxication there should take place not only a paralysis of the vessels but also transformations of their immunological condition. According to our hypothesis, the cells of the vessels should acquire an immunity to the toxin of the microbe that causes chronic infection.

The research of Galanova, Kravchenko, Matveev, Zamyri established the possibility for the cells of vessels in rabbits and in guinea pigs to attain immunity through active immunization by antigens Bac. abortus, Bac. typhi abdominalis, Bac. diphtheriae.

As has already been shown above, the botulinic toxin causes a strong contraction of the vessels in rabbits, guinea pigs and in the vessels of the human kidney. In chronic botulinic infections there should take place a transformation in the immunological condition of the cells. In order to study this problem, we also applied the Kravkova-Pisemski methodology.

Rabbits were infected subcutaneously, guinea pigs through the mouth, with sub-lethal doses of spores from the botulism bacillus type A and B that had been freed from toxin by means of heating in a water bath at 80 degrees over one hour of time. After the contamination with spores, the weight of the animals was registered; it appeared that they gained in weight identically with the control

group. Rabbits and guinea pigs were infedted by different amounts of spores. Determination of the amount of spores in one milliliter was conducted by the same method as in preceding experiments.

Tests on Rabbits

Over a period of six months, while the experiments lasted of 70 rabbits contaminated with spores only eight died; five of these from pneumonia and three from unknown causes. In six of the dead animals, the organs produced the botulism microbe although symptoms of the desease had not been observed. Of the 44 control rabbits in the same period, seven died; three of them from pneumonia and the remaining due to unascertained circumstances.

We studied the reaction of vessels in rabbits' ears to the dialysized toxin which was prepared in the above described manner. Prior to the test, the toxin was diluted 1:50,000 and 1:10,000. At the beginning the toxin was passed through the vessels in a stronger dilution and then in the weaker. After the toxin, the vessels were washed with the Ringer-Lokk solution until the reestablishment of their original condition. Eight tests were made on rabbits which can be divided into two groups on the basis of their results.

In the first group of experiments (Table 11) the rabbits were infected with 100 to 200 million viable spores, to which in the first two experiments 50 to 60 million were added daily and in the remaining experiments every three to four days.

The tests were set up between the third and 19th day after contamination of the animals. In experiment No. 1, the vessels in the ears of ten rabbits infected with spores reacted by expanding

to the first dose of toxin (1:50,000); the quantity of drops leaving them increased. For the second dose of toxin (1:10,000) the vessels in the ears reacted with a small contraction; the quantity of drops decreased by 7.4 percent on the average. In experiments No. 2 and 3, the vessels in the ears of 16 rabbits reacted with contraction to both doses of the toxin; the quantity of drops, when passing through the toxin diluted to 1:50,000 decreased on the average from 3.8 percent to 8.8 percent; the toxin in a dilution of 1:10,000 caused a decrease in the number of drops from 6 to 12.6 percent. In test No. 4 there were five rabbits, the vessels in the ears of which reacted by expanding to the first dose of the toxin but contracted after the second dose. In this experiment, the vessels of three rabbits reacted identically with those in the control group to the passing toxin. The vessels in the ears of the control animals in tests No. 1, 2, 3 and 4 (20 rabbits) reacted to both doses of the botulinic toxin by contracting. The amount of drops decreased on an average of 14.3 to 33.5 percent when the toxin was passed through.

On the basis of these experiments, we see that already during the third day after the infection of the rabbits with spores from the botulism microbe, the vessels in the ears of these animals react differently to the specific toxin than the vessels in the ears of normal animals. The vessels in the ears of normal rabbits react to the passing toxin with a clearly expressed contraction, whereas the vessels in the ears of rabbits infected by spores react to this very same toxin with a much weaker contraction or even with expansion.

In the second group of tests (Table 11) the vessels in the ears of 28 rabbits, between 41 and 47 days and even between 62 and 145 days after infection by spores from the botulism bacillus, reacted only with a slight contraction to the passing toxin of this microbe; the number of drops coming out of the ear vessels decreased on an average of from 10 to 20.8 percent. During the very same time the ear vessels of 17 normal control rabbits reacted with a stronger contraction to the toxin of this series and of the same dosage; the number of drops from them decreased between 14.7 and 42.7 percent.

Thus the vessels of the infected rabbits reacted to the same doses of botulinic toxin two to three times weaker than the vessels of normal animals.

In connection with the fact that botulinic toxin causes a strong contraction in normal vessels, the hypothesis arose as to whether the weaker reaction to the toxin by the vessels of rabbits infected with spores was not connected with their paralysis by the toxin that formed in the organism and precipitated the contraction. During the first period after infection with spores, this paralysis of the vessels apparently occurs. This is indicated by the irregular reaction to the toxin in tests No. 1 and 4, in which the vessels at the beginning of the experiment reacted by expansion to the weak dilution of toxin and contracted to the stronger one.

In order to explain the mechanism by which the botulinic intoxication functions upon the vessels of rabbits infected by spores, we set up tests using adrenal (Tables 12 and 13).

Table 11. Immunological Condition of Vessels in Rabbits' Ears during Symptomless Botulinic Infection

Number of test	Experiment						Control				Toxin series
	Dose of spores (in millions)	Day after infection on which experiment was started	Number of rabbits	Number of ears	Average percentage of decrease in drops when diluted toxin passes		Number of rabbits	Number of ears	Average percentage decrease in drops as diluted toxin goes		
					1:50,000	1:10,000			1:50,000	1:10,000	
1	100-200	3 & 4	10	16	Expansion	7.4	5	8	15.9	18.5	1
2	100-200	4 & 7	10	10	8.8	12.6	6	6	15.7	18.5	1
3	100-200	13 & 14	6	11	3.8	6.0	5	3	14.4	33.5	1
4	100-140	11 & 18	5	8	Expansion	18.6	4	6	14.3	16.6	2
	100-140	13 & 19	3	5	18.8	17.0	-	-	-	-	-
5	100-150	41 & 47	7	13	10.0	20.0	5	9	28.5	43.3	2
6	150-200	62 & 65	6	6	10.6	8.7	3	3	14.7	15.4	2
7	50-70	64 & 120	4	6	13.0	15.0	4	8	30.0	41.8	3
8	50-70	135 & 145	11	20	20.8	18.1	5	10	40.0	42.7	3
	Totals		62	95			37	53			

Table 12. Reaction of Vessels in Rabbits' Ears to Toxin & Adrenalin in Symptomless Botulinic Infection

Number in sequence	Number of rabbit	Dose of spores (millions)	Day after infection when experiment was begun	Reaction to the toxin							Reaction to adrenalin					
				Ringer-Lokk solution	1:100,000	Ringer-Lokk solution	1:50,000	Ringer-Lokk solution	1:10,000	Ringer-Lokk solution	Ringer-Lokk solution	1:100,000,000	Ringer-Lokk solution	1:1,000,000	Ringer-Lokk solution	
1	1	150-120	4	116	78	116	80	114	80	100	120	115	120	116	120	
2	2	ibid.	4	116	124	102	104	80	90	80	110	110	114	100	105	
3	3	ibid.	5	118	76	116	70	94	52	74	80	66	76	62	78	
4	5	ibid.	5	128	138	100	114	98	104	96	132	120	132	118	132	
5	6	ibid.	5	138	138	122	128	122	106	110	134	122	130	84	130	
6	4	ibid.	6	128	88	126	78	98	78	110	128	120	126	108	120	
7	8	ibid.	6	96	116	94	58	100	108	84	108	100	103	95	100	
8	7	ibid.	6	98	118	90	102	92	102	90	94	74	84	70	112	
9	9	ibid.	7	100	60	74	68	86	80	92	88	72	78	68	92	
10	11	ibid.	7	104	106	112	118	102	116	104	138	112	130	110	130	
	Totals			1142	1042	1052	920	986	926	940	1138	1011	1093	931	1119	
Average vessel contraction..					8.8%		12.6%		7.2%			11.2%		14.8%		
				Control rabbits												
1						104	84	90	70	80	104	88	96	80	92	
2						122	82	83	62	64	98	80	92	78	80	
3											112	96	98	80	90	
4				94	90	90	92	80	74	76	90	76	88	68	90	
5				92	74	80	66	88	62	74	132	124	132	120	132	
6				70	52	64	48	68	56	68	70	60	70	62	68	
	Totals			256	216	460	372	409	324	362	696	524	576	488	552	
Average vessel contraction ..					15.6%		19.2%		20.8%			13.5%		15.3%		

Table 13. Reaction by Vessels in Rabbits' Ears to Toxin and Adrenalin in Symptomless Botulinic Infection

Number in sequence	Number of rabbit	Dose of spores (millions)	Day after infection when experiment was begun	Reaction to the toxin							Reaction to adrenalin					
				Ringer-Lokk solution	1:50,000	Ringer-Lokk solution	1:10,000	Ringer-Lokk solution	1:1,000	Ringer-Lokk solution	Ringer-Lokk solution	1:1,000,000	Ringer-Lokk solution	1:500,000	Ringer-Lokk solution	
1	6	150-200	62	68	60	68	76	90	70	88	110	40	106	80	96	- 150 -
2	4	ibid.	62	94	80	94	70	94	72	94	106	74	100	76	96	
3	1	ibid.	62	126	120	120	114	122	90	114	78	38	74	40	70	
4	5	ibid.	64	96	90	96	96	98	94	96	92	53	90	30	78	
5	2	ibid.	65	144	140	114	110	112	116	116	138	126	190	100	190	
6	7	ibid.	65	120	88	120	120	120	115	120	120	84	118	86	110	
	Totals			648	578	642	586	636	557	628	644	420	678	412	580	
	Average vessel contraction				10.8%		8.7%		12.5%			34.8%		39.3%		
	Control rabbits															
1				96	70	96	76	84	42	66	86	46	78	56	70	
2				116	100	100	90	94	82	92	118	86	98	40	96	
3				130	122	130	110	120	100	115	104	72	104	66	104	
	Totals			342	292	326	276	298	224	273	308	204	280	162	270	
	Average vessel contraction				14.9%		15.4%		24.8%			33.8%		42.2%		

The reaction of the vessels was studied in a parallel way: one ear with toxin, the other ear with adrenalin. The intensity of the reaction to adrenalin by the vessels in rabbits' ears, contaminated with spores from the botulism microbe in the early and later period of the infection, and by the vessels of uninfected animals was the same.

On the basis of these experiments we assume that a weaker reaction to the toxin by the rabbit ears, infected by spores, possesses a specific immunological character. The presence of agglutinates in the serum of these rabbits indicates a development of an anti-bacterial immunity in them. Anti-toxins in the blood of the experimental animals were not discovered.

The animals were killed one or two days after studying the reaction of the vessels. Next a culture from the liver, spleen, and blood was made in a Tarocci habitat; in tests No. 5 and 7, this was done from the main part of the brain in order to discover the presence of the botulism bacillus in them. This microbe was found in the organs of all infected rabbits in tests No. 1, 2 and in the organs of a majority of animals in tests No. 3, 4, 5, 6 and 7. The botulism stimulant was not discovered in the organs of rabbits used in experiment No. 8. In the stains from the organs of certain animals, substantial gram-positive bacilli similar to the botulism microbe were discovered although the spores were absent. Determination of the microbe in the cultures was conducted by means of bacterioscopy and a neutralization reaction with anti-toxin serum.

These observations clearly show that the botulism stimulant may exist in vegetative form within the organs of animals while they are still alive. In such a case, if a long period of time has elapsed since contamination of the animals with spores, the colonization of the organism with this microbe decreases. In test No. 8 during 135 to 145 days after contamination, in not a single of the 11 rabbits was it possible to isolate the botulism stimulant from the organs (Table 14).

The research undertaken on 62 rabbits, infected with the spores from the botulism microbe, and 37 normal animals (see Table 11) shows that the vessels from the rabbit ears infected by spores provides a weaker reaction to the toxin of this microbe. This is explained by the fact that the microbe spores germinate in vegetative forms within the organism, and these forms produce toxin. The toxin paralyzes the vascular system causing contraction, and in rare cases expansion, of the vessels during the first period of infection. Further under the influence of new small quantities of botulinic toxin, produced in the organism, the cells of smooth muscles of vessels acquire immunity toward it and in this connection react in a weaker fashion to the toxin passed through than do the cells of vessels in normal animals.

We established a reaction for agglutination and neutralization of the toxin with the serum for all rabbits contaminated by spores. The reaction to agglutination with the botulism microbe was positive for the majority of rabbits. Beginning with the 11th day after infection, serum was given to the agglutination in dilution of 1:50, 1:100; 1:200, 1:500 an in certain cases 1:1,000 (on

the 41st day after contamination and after longer periods). The reaction of agglutination on 11 rabbits in experiment No. 8 was positive with a serum dilution of 1:400, 1:500, 1:800 and 1:1,000 (Table 14). Anti-toxin was absent from the serum.

Table 14. Colonization of Organs and Titr of Agglutinates in Rabbits infected with the Spores of Bac. Botulinus

Number of Experiment	Dose of spores (in millions)	Number of rabbits	Number of rabbits in which Bac.botulinus was found	Blood	Liver	Spleen	Brain	Day, after infection, on which agglutination reaction was set up	Number of rabbits in which the titr of agglutinates was observed											Number of rabbits in which the agglutination reaction was negative
									1:50	1:100	1:200	1:300	1:400	1:500	1:600	1:700	1:800	1:900	1:1,000	
1	150-200	10	10	0	7	9	0	3- 4	-	-	-	-	-	-	-	-	-	-	-	10
2	150-200	10	10	0	7	5	0	4- 7	-	-	-	-	-	-	-	-	-	-	-	10
3	150-200	6	5	0	3	2	0	13- 14	-	3	1	1	-	1	-	-	-	-	-	0
4	100-140	8	6	1	3	3	0	11- 19	1	2	-	2	-	-	-	-	-	-	-	3
5	100-140	7	7	3	4	5	1	41- 47	-	-	2	-	-	1	-	-	1	-	2	1
6	100-140	6	5	0	3	2	0	62- 65	-	-	2	-	-	-	-	-	2	-	2	0
7	50- 70	4	3	1	0	2	2	64-120	-	-	1	-	1	1	-	-	-	-	-	1
8	50- 70	11	0	0	0	0	0	135-145	-	-	-	-	2	2	-	-	2	-	1	4
Totals		62	46	5	27	28	3		1	5	6	3	3	5	-	-	5	-	5	29

In such a way, it was established through the foregoing experiments that when rabbits are infected subcutaneously with non-lethal doses of spores from the botulism microbe, colonization by this microbes occurs

in the organs of the animals; this precipitates a symptomless infection in them. The spores, finding their way into the organs, germinate into vegetative forms and produce toxin; during a long action by insignificant quantities of toxin, the cells of vessels acquire an immunity to it. Besides, agglutinates are produced in animals.

Experiments on Guinea Pigs

The tests with guinea pigs were undertaken according to the Kravkova-Pisemski method and were set up for the purpose of explaining the immunological transformations caused by the botulism microbe when it penetrated into the animal organs from the alimentary tract. The possibility of such an infection of the animal organs was established in the preceding experiments on mice.

A total of four tests were set up. The guinea pigs were infected through the mouth with the aid of a metal tube and by means of spores of the botulism stimulant, first of all heated in a water bath at 80 degrees for one hour. The quantity of living spores in one milliliter was established by the preceding method. After contamination, the animals were observed, checking the weight of the test and control animals. All guinea pigs were maintained in the same identical conditions. In the course of the whole experiment, the increase in weight of those infected with spores as well as the uncontaminated animals remained identical.

In the first test, eight guinea pigs were infected by a single injection of 50 to 70 million viable spores. One guinea pig died of pneumonia; the botulism microbe was extracted from its

organs. The remaining animals were killed on the 27th to 34th day, separating the rear half of the body, and tests were conducted by the Kravkova method in order to explain the reaction of vessels to toxin prepared in the same way as previously. As can be seen from Table 16, the vessels of guinea pigs that had been infected with spores provided on the average a weaker reaction to the botulinic toxin than did vessels of control animals which were not contaminated. When the toxin was passed through, the vessels of infected animals contracted 10.7 to 17.8 percent while the vessels of the control group contracted 24.2 to 32.2 percent, i.e. more than twice as strongly.

In the second test 10 guinea pigs were contaminated with 150 to 200 million spores, which were injected three times at intervals of three to four days. During 38 to 39 days after the infection, the reaction to the toxin by the vessels of these animals was investigated. In the first dilution of the toxin (1:50,000) the vessels reacted with an average contraction of 10.7 percent and in the second dilution (1:10,000) of 16.3 percent. The vessels of the five control guinea pigs for the same dilutions of the toxin reacted more strongly in their contraction: in its first concentration on an average of 23.5 percent and in the second of 29.8 percent.

The third experiment applied a one-time infection to 10 guinea pigs with 50 to 70 million spores. Prior to studying the reaction of vessels, two guinea pigs died from pneumonia. The vessels of eight guinea pigs on the 63rd, 64th and 75th day after the infection reacted to the toxin with a contraction of 7.7 to 14 percent on the average; at this time, the vessels of the five control animals

with the same doses of toxin contracted on the average between 37.8 and 40.8 percent, i.e. about three times as strongly as the vessels of the contaminated animals.

In the fourth experiment, the investigation centered upon the reaction to adrenalin by the vessels of six guinea pigs which were infected three times with 150 to 200 million spores. These tests were launched with the aim of explaining the reason for the weaker reaction to toxin on the part of guinea pig vessels contaminated by spores.

From Table 15 it is clear that the vessels of these animals, on the 34th, 39th and 43rd day after infection by spores and the vessels of control animals provided almost identical reactions of contraction to adrenalin. The vessels of the test guinea pigs gave a contraction on the average of 27.9 to 43.4 percent to adrenalin, whereas the vessels of the five control animals provided an average of 29.5 to 32 percent.

Table 15. Immunological Condition of Vessels in Guinea Pigs during a Symptomless Infection with Bac. Botulinus

Number of test	Test or Control	Number of animals	Dose of spores in infection by mouth	Day after infection on which test was started	Reaction to toxin - average contraction of vessels (in %)		Reaction to adrenalin - average contraction of vessels (in percent)	
					1:50,000	1:10,000	1:1,000,000	1:500,000
1	Test	7	50-60	27-34	10.7	17.8		
	Control	3			24.2	32.2		
2	test	10	150-200	38-39	10.7	16.3		
	control	5			23.5	29.8		
3	Test	8	50-70	63-75	7.7	14.0		
	Control	5			37.8	40.8		
4	test	6	150-200	34-43				
	control	5						

This data shows that the weaker reaction to the toxin by the guinea pigs, infected with spores, appears to be a specific characteristic. We assume that it depended upon the immune condition of the smooth musculature in the vessels of these animals in their relation to the botulinic toxin.

At the moment of arrangement for the experiments, according to the Kravkova method, cultures were taken from the organs of the guinea pigs that had been infected with spores. The cultures were made in different surroundings for discovery of the botulism stimulant. This was determined by using the same procedures as in the other tests.

In the organs of 28 out of 31 guinea pigs, the microbe of botulism was found. The bacillus was isolated in a large number of cases, and more often than from other organs, from the liver and spleen. With the serum of all guinea pigs, an agglutination reaction for the botulism microbe was undertaken; it was found to be positive in 24 animals for different dilutions of the serum (Table 16).

Observation of guinea pigs provided the same results as the tests on rabbits. These experiments represent considerable interest, since they indicate the possibility of changing the immunological condition of vessels when the spores of the botulism microbe penetrate through the walls of the intestines into the internal organs of animals.

Under circumstances where the quantity of viable spores is small, there develops a symptomless infection contributing to the

Table 16. Colonization of Organs and Titer of Agglutinates in Guinea Pigs, Infected By Mouth with Spores from Bac. Botulinus

Number of test	Dose of spores (in millions)	Number of guinea pigs	Number of pigs organs in which bac.bot found	Blood	Liver	Spleen	Intestines	Day after infections when agglutination reaction was started	Number of guinea pigs in which titer of agglutinates was observed											Number of guinea pigs in which agglutination reaction gave negative results
									1:50	1:100	1:200	1:300	1:400	1:500	1:600	1:700	1:800	1:900	1:1,000	
25	50-70	7	7	-	4	4	3	37 & 34	-	-	2	-	2	-	-	-	-	-	-	3
26	150-200	10	10	1	6	4	6	38 & 39	2	3	2	-	3	-	-	-	-	-	-	-
27	50-70	8	5	1	3	4	3	63 & 75	-	-	1	-	4	-	-	-	1	-	-	2
28	150-200	6	6	-	5	2	2	34 & 43	-	-	-	-	2	-	-	-	1	-	1	2
	Totals ..	31	28	2	18	16	14		2	3	5	-	11	-	-	-	2	-	1	7

production of the botulinic toxin in small doses. Chronic intoxication in guinea pigs as well as rabbits causes the development of an immunity by cells of the vessels in the smooth musculature toward toxin; in this connection, when its solutions are passed through, they provide a weaker reaction than do the vessels of normal animals.

Apart from this, in guinea pigs as well as in rabbits the symptomless botulinic infection is accompanied by the production of agglutinates. Proof of existence of an infection lies in the fact that the botulism bacillus is found in the organs of a majority of the animals at the moment Kravkova-type experiments are arranged and during a long period of time, after the infection.

In these tests, as in the previous ones, the spores of the botulism microbe after their penetration into the organs of animals were not found there in the category of extraneous dead substrata that do not manifest any activity. On the contrary, this was an infection with manifestations of activity on the part of a micro-organism; as a result of this, in the organism of guinea pigs there took place substantial immunological transformations. Agglutinates were discovered in animals, the reaction of cells of vessels to toxin changed (it became weaker), the microbe of botulism was isolated from the organs.

In this connection, the infection of guinea pigs took place without any inner manifestations (the animals ate fodder well, gained weight on a par with the control group, death from botulism was not observed). On this basis, we assume that it was symptomless, chronic. It is important that the development of this infection in guinea pigs

was established through infecting them with spores by mouth as well as through subcutaneous contamination.

4. Reaction of the Smooth Musculature in Experimental Animals during a Symptomless Infection

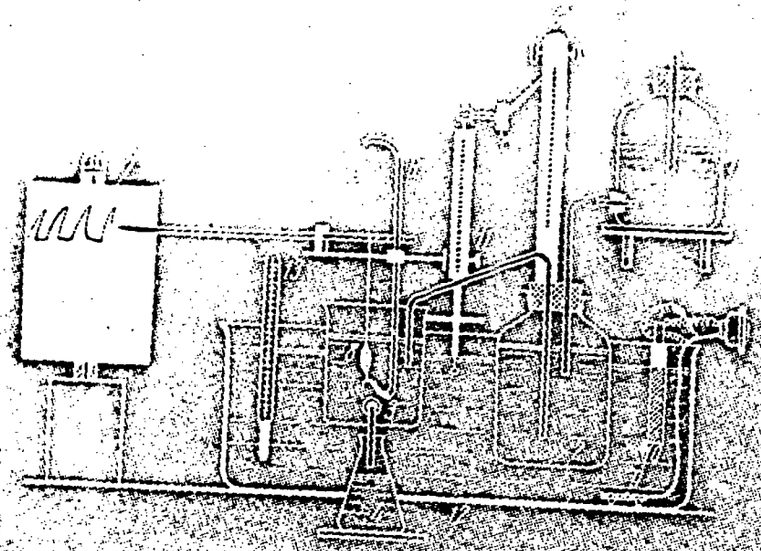
Our previous observations devoted to the study about the condition of cells in vessels of rabbits and guinea pigs during a symptomless botulinic infection showed that the spores of this microbe introduced internally or parenterally into the organism of animals causes changes in the reaction of cells in vessels to the botulinic toxin.

In connection with these observations, the hypothesis arose that the cells of other organs also undergo immunological rebuilding under the influence of small doses of botulinic toxin, produced in the organs of animals.

Our further research was undertaken with the aim of studying the immunological condition of cells in the organs of animals during different periods, after they had been contaminated with the spores from the botulism microbe. Basing ourselves on the work in the field of cellular immunology by Galanova (1934), Zamuri (1936), Matveev (1937), Kravchenko (1938) and Akonyan (1938) we turned our observation to the reactive ability of the uterus and the intestines of guinea pigs, infected with sublethal doses of spores from the botulism microbe, when they were being affected by the activity of the dialysized toxin of this bacillus.

Figure 3. Schemat of a Schultz-Delia Apparatus

1 - bottle with Ringer-Lokk solution; 2 - vessel with Ringer-Lokk solution, preserving constant temperature; 3 - small glass, where beak of uterus or fragment of intestine is located; 4 - vessel for fluid, leaking during the washing of organ; 5 - Schwartzman stative; 6 - glass tube with hook, to which organ is tied; along this tube, air is forced through a water-jet pump into the organ; 7 - cylinder of kymograph with smoked paper; 8 - isolated organ in small glass; 9 and 10 - electric heater with thermoregulator; 11 - regulating ligature; 12 - tub with water; at all times in test, a constant temperature of water is maintained which is heated electrically with a thermoregulator; in our tests, a small glass with the isolated organ was submerged in the tub; 13 - thermometer, controling temperature of water in the tub.



The action of the extracts (toxins) of the smooth paracybic platheminthes upon the isolated intestine was studied by Talyzin (1941); according to his observations, concentrated solutions precipitated a short-time stifling of the rythmic functioning of the intestine after which a stable increase in tonicity occured.

Petrovski, Naumenko and Baturenko (1937), Shkavera and Serebryanaya (1941), studying the action of the botulinic toxin upon the smooth musculature of the isolated intestine, noted the stimulating property of this toxin. Apart from this, Shkavera and Serebryanaya observed an increase in the contraction of the uterus beak from a female rabbit under the influence of the botulinic toxin.

Research on the reactive capacity of the uterus in the intestine of guinea pigs, when toxin was affecting it, was conducted by us with the aid of the Schultz-Delia methodology applied by these authors for the study of cellular immunity.

The Schultz-Delia apparatus is schematically presented in Figure 3.

Before the experiment, the primeval guinea pigs were killed. The stomach cavity of the animal was opened, the uterus carefully separated from the fat, and the beak at the base of the uterus cut off with shears. All of this has to be accomplished with great care so as not to wound the organ, since this could affect the results of the test.

One uterus beak remained in a small glass with a Ringer-Lokk solution heated to 37 degrees; air was introduced into the small glass through the glass tube at all times. Simultaneously, the

other uterus beak underwent a test in the Schultz-Delia apparatus. The uterus beak was placed in a small glass with a 50 milliliter capacity, where it was tied to the glass hbok having an opening to supply air. The other end of the uterus beak was attached to a lever, which had a pen at one end for tracing the curve on the cylinder of the kymograph.

The small glass, in which the organ was located, was filled with the Ringer-Lokk solution heated to a temperature of 37 degrees; air was supplied at all times into the small glass through the fractured hook to which the uterus beak was fastened. After placing the uterus beak in the small glass, it remained quiet for a period of 20 to 30 minutes. During this time, the contractions of the organ were taking place at the slowest possible rate. When the organ had been in a quiet condition for 5 to 10 minutes, the experiment was started. The pen-lever, to which the uterus beak was attached, was led to the cylinder of the kymograph with the smoked paper; the botulinic toxin in a dilution of 1:100 was poured into the small glass. The toxin was prepared in the same way as for the experiments on the vessels. After the activity of the toxin had been completed, the pen-lever was removed from the cylinder which was halted; the uterus beak was then washed in a Ringer-Lokk solution until the organ was completely quiet. After this when the uterus beak ceased to contract, the pen-lever was again brought to the revolving cylinder of the kymograph; and a new dose of toxin was poured into the small glass. The washing process was conducted under the condition that ~~that~~ the uterus beak did not contract from the effect

of the toxin. The doses of the toxin were increased at all times, but they did not go over two to three milliliters.

On the isolated intestines of guinea pigs, tests were also carried out in the Schultz-Delia apparatus. The guinea pigs were killed, the stomach cavity opened, and the middle part of the thin intestine taken for the experiment. The latter was carefully separated from the mesentery. At the end of this intestine fragment, ligatures were placed, and it was lowered into a small glass with a Ringer -Lokk solution heated to 37 degrees. Air was admitted at all times to the intestine in the small glass.

With the establishment of a normal peristalsis of the intestine, a fragment two centimeters long was taken from it for the experiment. This fragment of the intestine was attached to the glass hook and to the lever of the pen, just like the uterus beak. The small glass in the apparatus was filled with a Ringer-Lokk solution, the intestine was lowered into this, and air was at all times given access to the small glass. The pen-lever was led to the cylinder of the kymograph having the smoked paper in order to record the rythmic contractions of the intestine. At the beginning a small dose of the toxin was added to the glass, then twice as large a dose, etc. After adding each new dose of the toxin, the fragment of intestine was washed with the Ringer-Lokk solution. In view of this fact that the initial rythm for contraction of the intestine was resumed quite rapidly after washing it from the toxin, in the course of the experiment unbroken tracings of the curve were maintained on the cylinder.

In order to determine the viability of the isolated organs (uterus and intestines of guinea pigs), after the action upon them of various botulinic toxin doses, they underwent irritation by the endotoxin of B. typhi abdominalis.

The endotoxin of this microbe was prepared in the following manner. The bacteria were cultivated in hard surroundings for 24 to 48 hours, after which they were washed in a physiological solution, and a suspension was prepared from them. The suspension comprised 20 milliard microbe bodies in one milliliter; The microbes were killed through heating at 60 degrees for two hours. Then the suspension was checked for sterility by means of cultures in various surroundings and was preserved in a refrigerator at 2 to 3 degrees.

Primeval guinea pigs weighing 200 to 250 grammes were infected subcutaneously with such doses of spores from the botulism microbe that the death of the animals would be at a minimum or totally absent. Besides, the infection caused by this quantity of spores should not have influenced the condition of the animals: increase in weight and preservation of appetite. Liberation of the spores from toxin took place by washing them in a physiological solution and heating them in a water bath at 80 degrees over a period of one hour. The quantity of living spores in one milliliter was determined by the same method as in preceding tests.

In the first series, 16 guinea pigs were infected with 100 to 140 million living spores from the botulism bacillus type A and B. The contamination took place twice with 50 to 70 million over

a period of three days. There were six control, uncontaminated guinea pigs. The latter were maintained under exactly the same conditions as were the test animals. During seven days after the first contamination, one guinea pig died from botulism and another one from an unknown cause. After the infection, the guinea pigs preserved a good appetite and gained in weight. The average weight prior to contamination was equal to 307.1 grammes, whereas after the infection on the day tests were made it was 371.2 grammes. The weight of the control guinea pigs before the experiment made an average of 292.6 grammes, while on the day when the reaction of the organs to the toxin was observed it reached 363.3 grammes. So the test and control animals gained weight evenly. At the time of the experiment with the uterus and intestine of animals in the Schultz-Delia apparatus, a culture was taken from the other organs in order to determine the presence of the botulism microbe in them. In the blood of these guinea pigs, the botulism stimulant was discovered three times, in the liver 11 times, in the spleen nine times, and in the main part of the brain five times. With the serum of the animals, a reaction for agglutination with a culture from the botulism bacillus was undertaken. A positive reaction was given by the serum of 11 guinea pigs in a dilution of 1:10 to 1:100, and only in one case at a 1:200 dilution. No anti-toxins were discovered in the serums of the guinea pigs.

During the infection with sub-lethal doses of spores from the botulism bacillus, the guinea pigs did not manifest any phenomena observably connected with the desease and normally increased

their weight in no way differently from the control animals. On the basis of the foregoing, we assume that the infection was symptomless.

Investigation of the reaction by the uterus and intestine of guinea pigs in the Schultz-Delia apparatus to the toxin was conducted on the 8th to 24th day after the first infection. Beginning with the eighth day after the contamination, one or two tests were made on the organs of the animals. The botulinic toxin was applied for the experiments, in a minimum lethal dose which equaled 0.00001 gramme for mice.

Into the small glass, which contained the organ, alternately 0.5; 1; 2; and 3 milliliters of the dialysized toxin was added. The toxin had been diluted 100 times in a physiological solution. It remained in juxtaposition with the organ for two minutes, since we had noticed that during such a period of time the most powerful contracting action takes place upon the cells of the smooth musculature in the uterus and intestine of guinea pigs.

To check on the viability of the organs after the botulinic toxin, their reaction was investigated with reference to endotoxin B. typhi abdominalis which was added to the glass also in quantities of 0.5; 1; and 2 milliliters. The action of this endotoxin upon the uterus usually lasted 40 seconds and upon the intestine - one minute.

Comparing the curves (Figures 4 and 5, 6 and 7), reflecting the reaction to the toxin of the uterus and intestine of an uncontaminated guinea pig, and the reaction to toxin of these same organs

in a guinea pig on the 16th day after its infection with spores from the botulism bacillus, we see that these organs of the latter animal react more strongly to the toxin by contraction than do the organs of the former.

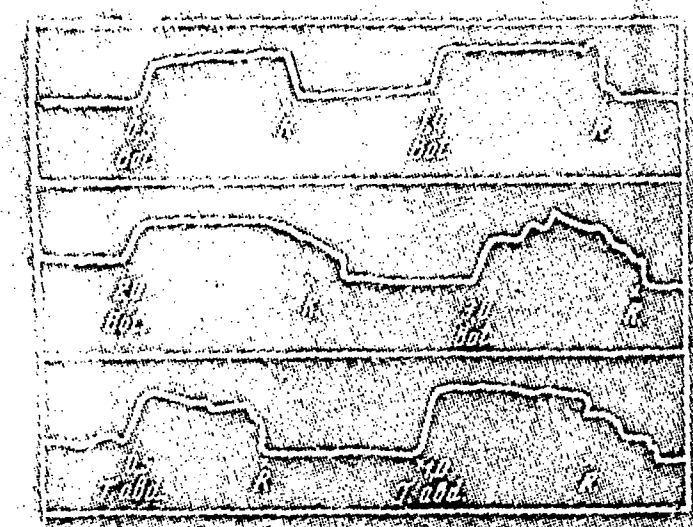
The organs of the guinea pig, contaminated with spores from the botulism microbe, possess a higher sensitivity to the toxin of this bacillus.

Figure 4. Contraction of the uterus beak in a guinea pig, not infected with spores of the botulism bacillus, during action by the toxin of Bac. Botulinus and the endotoxin B. Typhi Abdominalis.

Figure 5. Contraction of the intestine in a guinea pig, uncontaminated by spores from the botulism bacillus, during action by the toxin of Bac. Botulinus and the endotoxin of B. Typhi Abdominalis.

Figure 6. Contraction of the uterus beak in a guinea pig, infected with spores from the botulism bacillus, during action by the toxin Bac. Botulinus and the endotoxin B. Typhi Abdominalis.

Figure 7. Contraction of the intestine in a guinea pig, contaminated by spores from the botulism microbe, during action by the toxin of Bac. Botulinus and the endotoxin of B. Typhi Abdominalis.



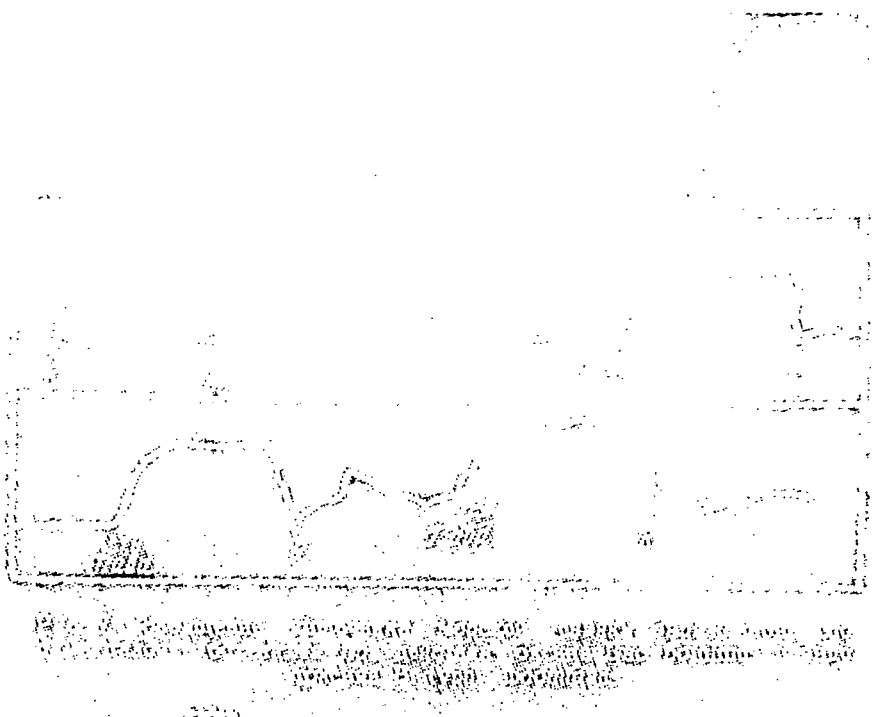
Reaction of organs to toxin of Bac.Botulinus

Reaction of organs to endotoxin B. Typhi Abdominalis

Figure 8. Average height of curves for contraction of uterus in guinea pigs during 8-24 days after infection with the spores of Bac. Botulinus.

Figure 9. Average height of curves for contraction of intestines of guinea pigs during 8-24 days after infection with the spores of Bac. Botulinus.

Figures 8 and 9 demonstrate the average contraction force during the action of the toxin Bac. Botulinus upon the cells of the smooth musculature in the uterus and intestines of all guinea pigs in the first series of tests which had been infected with the spores of this microbe. As was shown, this contraction was much stronger than the contraction by the musculatures of these organs in the normal control animals (Figures 10 and 11). The heightened sensitivity of the uterus and intestines toward the toxin was discovered in 12 guinea pigs (out of 16 infected by the spores), and in some of the animals these organs reacted to the toxin similarly to the organs of the six normal control guinea pigs.



Reaction of organs to toxin of Bac.Botulinus

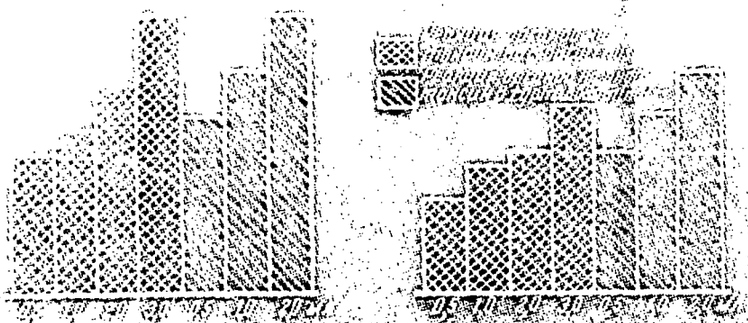
Reaction of organs to endo-toxin B. Typhi Abdominalis

Figure 10. Average height of curves for contraction of uterus in normal guinea pigs.

Figure 11. Average height of curves for contraction of intestines in normal guinea pigs.

A stronger reaction on the part of organs in animals infected by spores depended upon whether the spores germinated into vegetative forms within the organs, which forms then produced toxin in small quantities and through the toxin sensibilized the cells of the organs. However, in so far as the toxin was produced in insufficient amounts to cause the botulism desease in animals, on the surface no manifestations were visible. Apparently the toxin was also produced insufficiently for the formation of considerable amounts of anti-toxin, which was not found in a majority of cases in the blood of guinea pigs.

In the second series of tests, 15 guinea pigs were contaminated with 150 to 200 million spores of Bac. Botulinus; four guinea



pigs were used for the control group. The spores were injected subcutaneously three times with 50 to 70 million each time at intervals of six days. Prior to the establishment of the tests, three of the guinea pigs died from botulism and one from an unknown cause. Despite the large dose of spores, the guinea pigs (and the control group also) increased in weight. No symptoms of the desease were observed on them. Investigation of the reaction by the uterus and intestines to the toxin of the botulism bacillus was conducted at different periods, during 45 and 85 days after the contamination.

Depending upon the reaction of the organs to toxin, the animals in this series of tests were divided into three groups: 1) with heightened sensitivity of the uterus and intestine - four guinea pigs; 2) with a very weak reaction of the organs to toxin which can be looked upon as the beginning in the development of immunity, or giving completely no reaction thanks to the immunity produced - four guinea pigs; 3) with such a reaction of the organs to toxin as that in the control, normal animals - three guinea pigs.

During a period of 45 days and longer after infection with the spores, the animals still manifested a heightened sensitivity in the cells of their internal organs toward the botulinic toxin; and furthermore, under the influence of new toxin doses from the germinating spores, immunity begins to develop.

Only in such a way, apparently, is it possible to explain the differing reaction of the animal organs to the toxin in this series of experiments.

In the cultures from the organs of guinea pigs in the second series of tests, the botulism microbe was discovered in the blood twice, in the liver five times, in the spleen four times, and in the main part of the brain twice. The bacillus was not isolated from the organs of three pigs. The agglutination reaction with the botulism microbe was positive for certain guinea pigs in a dilution of 1:400, and in certain others 1:1,000. Anti-toxin was discovered in the blood of two guinea pigs in an insignificant quantity; 0.5 milliliter of serum neutralized 2 Dlm of the botulinic toxin in these animals. The organs of these guinea pigs provided a very weak reaction to the toxin during their observation in the Schultz-Delia apparatus.

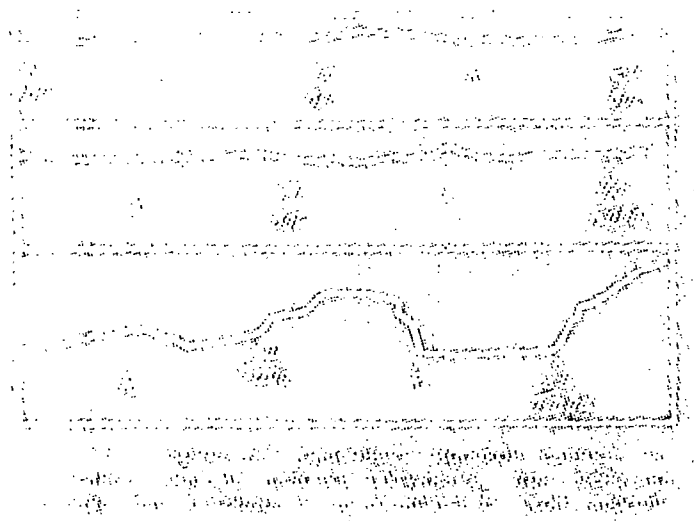
In the third series of experiments, the reaction to toxin by organs of animals after a long period from their infection was studied. For this purpose, ten guinea pigs were contaminated with 100 to 140 million viable spores from the botulism bacillus which were introduced twice from 50 to 70 million each time at intervals of five days. Besides that, five guinea pigs were left uninfected as a control group. Prior to the arrangement of the test with the isolated organs, one guinea pig died from botulism. The remaining ones, identically like those used for control, gained weight very well. Symptoms of the desease were not observed in them. The blood of all guinea pigs, taken on the day the tests were set up, showed agglutinates to the botulism microbe at a serum dilution of 1:[illegible], 1:500, and in some 1:1,000. The serum of two guinea pigs in a quantity of 0.5 milliliter neutralized 2 Dlm of the botulinic toxin.

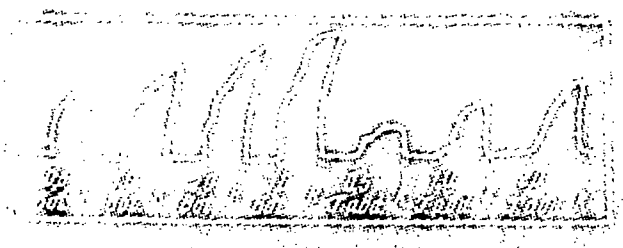
In the cultures from the organs of guinea pigs, the microbe of botulism was discovered in the blood once, in the liver four times, in the spleen twice, and in the main part of the brain once. In the organs of four guinea pigs, the botulism microbe was not found at all.

The study of the reaction by the uterus and intestine of animals toward the dialysisized toxin was conducted on the 92nd day; the very latest tests were made on the 100th day after the first infection. The curves on figures 12 and 13 show that none of the four toxin doses caused any contraction by the organs of guinea pig No. 7 which had been infected with spores. At the same

Figure 12. Contraction of the uterus beak in a guinea pig infected with the spores from botulism bacillus, during the action of toxin Bac.Botulinus and endo-toxin B. Typhi Abdominalis.

Figure 13. Contraction of the intestine in a guinea pig infected by spores from the botulism microbe, during the action by toxin Bac. Botulinus and endotoxin B. Typhi Abdominalis.





time, the organs in the control guinea pig No. 4 gave the usual reaction to the toxin observed in normal guinea pigs during the course of all tests (Figures 14 and 15).

Figure 14. Contraction of the uterus beak in a guinea pig, not infected by spores of the botulism microbe, during action by the toxin of Bac. Botulinus and endotoxin B. Typhi Abdominalis.

Figure 15. Contraction of the intestine in a guinea pig, not infected by the spores of botulism bacillus, during action of toxin Bac. Botulinus and endotoxin B. Typhi Abdominalis.

On Figures 16 and 17, the given diagrams portray the average power (in centimeters) of contraction by organs (uterus and intestines) in all nine guinea pigs of the third series of experiments. As we can see, the first three doses of the toxin cause a very insignificant

Reaction of organs to toxin Bac. Botulinus

Reaction of organs to endotoxin of B. Typhi Abdominalis

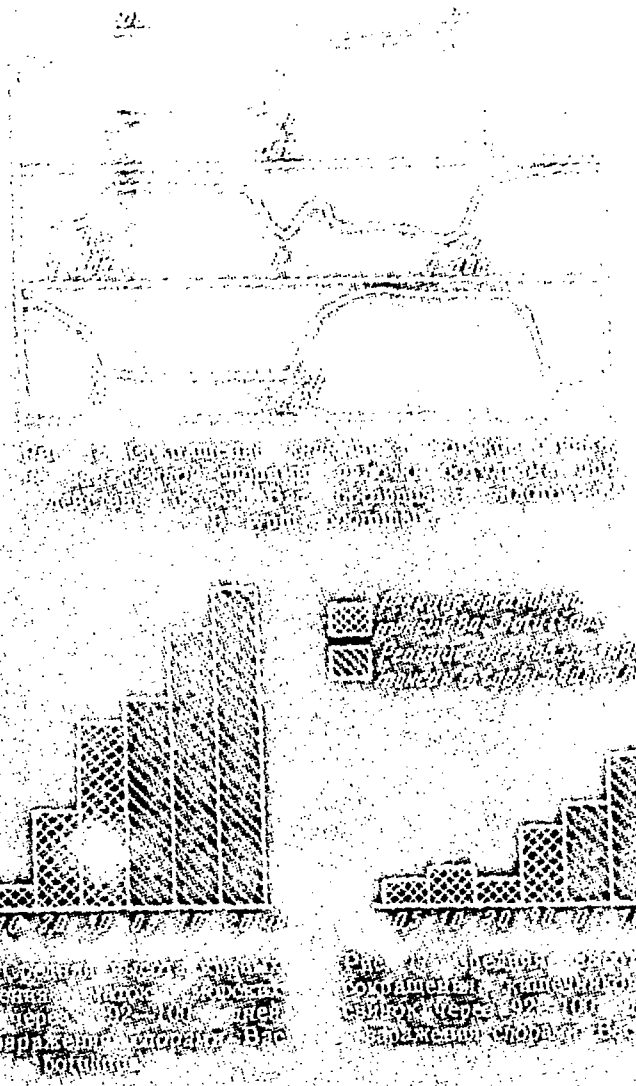
Figure 16. Average height of curves for contraction of uterus in guinea pigs during 92-100 days after infection with spores of Bac. Botulinus.

Figure 17. Average height of curves for contraction of intestines in guinea pigs during 92-100 days after infection with spores of Bac. Botulinus.

contraction and only the fourth dose (3 milliliters) precipitated a stronger reaction. However it was considerably weaker than in the control guinea pigs, not infected by the spores of the botulism stimulant (Figures 10 and 11).

In the majority of animals with symptomless botulinic infection, during 92 and 100 days after their infection by spores, an immunity of the cells in smooth muscles of the uterus and intestine toward the toxin of the botulism bacillus was created. The presence of immunity was indicated by the very weak reaction of their organs to the toxin of that microbe. The intensity of this immunity was, however, relative: the uterus and intestine of these animals, when applying larger doses of the toxin, caused a contraction.

From the three series of experiments that have been described, we see that during the infection of guinea pigs with the spores of



botulism bacillus, freed of the toxin, a colonization of the organs in these animals is observed by this microbe over a protracted period of time.

Most often of all the botulism microbe was discovered in the liver and the spleen: in six guinea pigs, it was isolated also from the blood of the heart and in eight guinea pigs from the main part of the brain. The presence of the microbe in the organs of animals did not precipitate any reaction, even from the direction of the central nervous system. The infection in general did not affect the condition of guinea pigs that had a good appetite, and they gained weight just like the control animals. At the same time, the infectious process developed very slowly within the organism. This is supported by the presence in the blood of the animals of agglutinates to the botulism bacillus. Antitoxins in rare instances may, apparently, accumulate in considerable quantity. They were discovered in the blood in small amounts all told only in four guinea pigs. This testifies to the fact that in the organism of animals the formation of toxin takes place in very insignificant quantities, although these are completely sufficient to cause changes in the immunological condition of cells in organs including the smooth musculature of the uterus and intestines.

There was not enough of the toxin, produced by the vegetative cells of the botulism bacillus, for the formation of considerable quantities of anti-toxin. During the first period of the infection, the toxin precipitated the sensibilization of cells in the organs of animals which gave a stronger contraction during

the action by the toxin of the botulism microbe than did the same organs in normal animals. In certain guinea pigs, the reaction of organs to the toxin of this microbe remained unchanging. This was in connection with the beginning in the development of immunity, which in the given instance brought about a loss in the heightened sensitivity to the toxin. During a lengthy course in the symptomless botulinic infection, the condition of increased sensitivity by the cells in the organs of animals took place gradually, and an immunity was developed. For this reason, the organs of guinea pigs during 92 and 100 days after infection with spores gave a much weaker contraction during action by the toxin of the botulism microbe than did the organs of the control animals. The immunity of the cells in the smooth musculature of the uterus and the intestine developed in all animals, despite the absence of the anti-toxin from the blood of the majority. This corresponds with the data, presented in the work of Kravchenko on the immunity of cells as well as in our research (1940) which showed that the immunity of cells in the organs does not depend upon the presence of anti-bodies.

The development of an immunity in the cells of organs in guinea pigs took place during a single disintegration of the botulism microbe in the organism. In the first series of tests, which were conducted during 8 to 24 days after the infection, the microbe of botulism was discovered in the organs of all animals. In animals on the organs of which tests were made substantially later, it was impossible to find the botulism bacillus; it was absent in three guinea pigs of the second series and in four guinea pigs of the third series of

experiments. This phenomenon was observed in our previous tests on mice and rabbits: during a long period of time after the infection with spores, no botulism microbe was to be found in their organs either.

The data from this research shows that the presence of spores from the botulism microbe in the organs of animals does not appear to be differentiated for it. The colonization of the organism with spores - this is not an inculcation of particles, it is an infection during which the botulism microbe lives in the organs, germinates into vegetative forms, and causes the formation of agglutinates, and at times also anti-toxins; the action by the toxin of this microbe conditions a deep immunological transformation in the cells of the animal organs. All of this supports the contention that botulism appears to be toxic-infectious and that it takes place very sluggishly in those circumstances when the spores penetrate into the organism but very violently when these are accompanied by the toxin. When the animal organism is infected (apparently also in the case of a human being) by spores from the botulism microbe, the toxic-infection takes place along the lines of a symptomless infection with corresponding immunological changes in the blood and organs.

C H A P T E R V

CLINICAL PICTURE OF THE BOTULINIC INTOXICATION IN THE HUMAN BEING

The general clinical symptoms of botulism were described long before the discovery of this deseases stimulant. In pre-revolutionary Russia, in connection with the many poisonings from fish, the clinical course of botulism was described quite thoroughly in the works of Anrep (1885), Berkovski (1857), Sokolov (1896), Chugin (1883), Chernyshev (1889), Arustamov (1891). In other lands, primarilly in Germany, botulism was caused by venison and sausage, and was described by Mueller, Guzeman, and others.

In 1895, Van Ermeng also wrote about the clinical aspects of botulism and revealed the role of toxin in the appearance of many syptoms of the poisoning. Van Ermeng corroborated his clinical observations by means of experimentation with the toxin on animals.

In subsequent experimental tests and clinical observations it was established that the symptoms of botulism are caused by the poisonous action of the toxin upon the cells of the human and animal organisms.

The toxin may enter the organism together with food products, as a result of botulism microbes developing in the latter, orik it may be produced by this microbe when it already exists in the organism of human beings or animals. In this connection, a large role is played by the first small potions of the toxin that weaken the defensive capabilities of the organism.

Related to the fact that the cells in the nervous system are paralyzed by the botulinic toxin more than the cells of other organs is the fact that the syndrome of botulism fundamentally consists of a complex of nerve-paralytic phenomena, which express themselves in an upset of innervation of the muscular system. Usually symmetrically general or local paralysis is observed. The general disorders are manifested by increased or decreased secretion and motor functions in the initial part of the alimentary duct. In this connection dryness is observed in the mucous membrane of the mouth nodule, at times salivation, and also appearances of dysphagia. At the beginning of the illness almost always there occur nausea, vomiting, pain under the pit of the stomach, all of which indicate dispeptic phenomena.

The incubation period differs: most often of all it is from 18 to 24 hours, but at times two to three days. The described cases of botulism have had very short incubation periods - between two and six houbs, as well as very long ones - from nine to ten days. In some botulism epidemics, relapses from the disease were observed after a full recovery.

The varying length of the incubation period depends upon the the very first dose of the toxin which has entered the organism with the food product, and also depends upon the immunological condition of the human organism or that of the animal: the larger the initial dose of toxin, the shorter the incubation period; the more intensive the anti-botulinic immunity, the longer the incubation period.

Apart from this, according to our hypothesis, a long incubation period in the human and animal is observed under such conditions

when the initial dose of toxin entering the organism withbthe food is very small; in such circumstances the basic role in the development of symptoms is apparently played by the toxin, produced in the organism by the botulism microbe.

According to the data of Mirtovski and Govseev (1937), a direct relationship exists between the length of the incubation period and the lethalness. A light case of the disease is preceded in most cases by an incubation period of four to five days. Certain authors note the considerable number of botulism cases with a very slow development of the disease, in which the incubation period lasted up to thirteen 24-hour periods (Nikolenko and Kamenskaya, 1937; and others). Apart from this, all researchers emphasize the very slow recuperation of botulism patients.

During the past 25 years, botulism epidemics have been described many times in the USSR and also in other countries. We will not stop to write about each epidemic as a separate item but will consider only the works of those authors who studied the disease in detail or those who describe completely new phenomena on the clinical aspects of botulism.

In the writings of foreign authors, devoted to this question, only the general clinical picture is usually cited without a detailed description of the syndrome of botulism. World literature about botulism contains only one work which provides a very detailed study of the epidemology, pathogenesis and clinical picture of botulism. This book was written by Soviet doctors who studied botulism at the time of the epidemic in Dnepropetrovsk of 1933, which had been caused

by fruit preserves. Medical dostors with different specializations participated in the observations, and the results of this collective work was of considerable importance for the correct understanding of the pathogenesis and clinical aspects of botulism.

1. Peptic Disturbances during Botulinic Intoxications

When using food products containing the toxin and microbes of botulism, the alimentary canal is the first to come into direct contact with the latter. Koritski and Meier reported the hypothesis that the phenomena of gastro-enteritis, which were noted by many authors, develop with botulism in connection with the irritating action not by the toxin but by the bacteria. On the other hand, the symptoms caused by the action of the botulinic toxin do not develop at once in the alimentary canal but only after a varying incubation period.

Koritski (1938) in his research on the peptic disturbances attending botulinic intoxications, conducted on 272 cases of botulism, notes that the symptoms arising when the alimentary canal is stricken by the toxin, in the majority of cases, arise simultaneously with other symptoms. The work of Koritski, in the quantity of material and the carefulness of his study, appears to be the only literature in the world of this type on botulism, and that is why we shall go into it in great detail.

The first group (72 men) comprised patients ill with eroded as well as uncertain forms of botulism. Disturbances of the alimentary tract were manifested in this group through pain in the intestines, dryness in the mouth and throat. Disruption of deglutition

in the patients of this group were found less frequently. Nausea and vomiting were not often observed (in 26 to 29 percent of the cases). The bowel movement was normal for the majority of the patients.

The second group of patients consisted of 53 with a light form of botulism. Koritski notes that this form of the disease is identified by the differentiation in its symptoms. Dryness in the mouth, nose and pharynx appeared on the third or fourth day of the illness and was observed in 65 percent of the patients. In 12 percent, a copious secretion of saliva was noted. Difficulties in deglutition and dysphagia as a result of spastic phenomena were observed in 44 percent of the patients. In a majority of cases, these symptoms appeared during the first days of the disease; at times on the third to eighth day during the peak in the development of the infectious process. In more than one-half of the sick persons there was nausea; vomiting was observed only in 24 percent of the patients. Among the symptoms indicating paralysis of the intestinal tract were pains in the stomach area and intestines, which usually appeared during the first days of the illness.

The third group consisted of 52 persons sick with a heavy form of botulism, ending by a return to health. The disease in these patients was also accompanied by a number of symptoms. Especially sharply expressed was the paralysis of the nerve-muscular apparatus. Dryness in the mouth and pharynx was observable in 70 to 75 percent, the phenomemnon of salivation - in 14 percent of cases. The author emphasized that the earlier dysphagia appeared,

the harder the desease. Dysphagia was accompanied by difficulties in breathing. Difficulties were also observed in the masticatory muscles' functioning, a sharp muscular weakness, and hanging of the lower maxillary (at times the mouth had to be closed with a hand). Vomiting was noticed in certain of the patients; many of them manifested flatulence, with visibly expressed constipation. Pains in the area of the stomach and intestines were noted in 57 percent of the cases.

The fourth group, in which there were a considerable number of lethal cases from botulism, comprised 92 patients. Of these, 75 were studied. However, even this number was not studied in detail due to the seriousness of their condition. Sharply expressed symptoms of paralysis in the nervous and muscular systems were noted in them, as well as the remaining symptoms observed in the preceding group. In all cases, there was a progessive disturbance in swallowing with the appearance of a stable aphonia. With the growth of the symptoms, a sharp paleness of the skin and the mucous glands was observed; this can be explained through the contraction of the blood vessels under the influence of the toxin. In order to characterize the clinical picture for this form of botulism, we will cite one case history of the disease from the work by Koritski.

"Z.G., 24 years old, during eight 24-hour periods after poisoning she suddenly felt her head spinning at work, saw darkness in front of her eyes, objects seemed doubled, and was nauseated. A pain started in the area under the stomach pit. In the evening, she came to the hospital herself. On the second day, the same phe-

nomena. On the third day, pressure and heaviness on the chest, ptosis of both eye-lids, disturbance of the convergence, difficulty in swallowing and speaking, shortness of breath, chilling of the extremities. On the fourth day, the state of health has somewhat improved, the voice is hoarse, but speech is clearer, swallowing takes place with difficulty, heaviness behind the chest, and difficult breathing. Head-ache, dryness in the mouth, heaviness throughout the whole body, sleepiness, but she can not sleep. Toward the evening, nausea, panting, speech is disorganized. On the fifth day, a sharp paleness of the integuments. The neurological symptoms increase: ptosis of both eye-lids, convergence disturbed, pupils of the eye react weakly to color. Swallowing is sharply difficult, nausea and calls to vomit. In the evening sharply worse condition, superficial breathing, non-rythmic, more difficult. Aphonia, aphagia, cyanosis. At twelve o'clock in the night - death with indications of asphyxiation."

The cited example shows that after a long period of infection, a serious illness with a lethal end may develop. Koritski considers that the length of the incubation period should be explained by the slow multiplication of the botulism bacillus in the organism. The course of many observed cases of botulism testifies, in the opinion of the author, to the toxic-infectious character of the disease. The author emphasizes in this connection that the toxin paralyzes not only the nerve but also the muscular system, and appears to be thus a strong miotropic poison.

2. Disturbances in the Functions of the Upper Respiratory Tract

The functions of the upper respiratory channels are also upset in botulism. The symptom which occurs most often, when the breathing channels are stricken, is the spasm of the larynx, dryness of the mucous glands, difficulty in breathing.

Halperin (1937) studied the condition of the respiratory channels in 81 persons sick with botulism, of whom 20 were males and 61 females. The author discovered a decrease or increase in the size of the cavities of the nose, in connection with the change in the blood content. The nose channel were enlarged or strongly reduced in size. The patients complained of pain in the nose and loss of smell. In 23 of the patients, a paralysis of the soft palate was observed; in 16 patients - disturbance of the motor functions and anemia of the mucous larynx which strongly interfered with breathing.

There was a lack of symptoms from the direction of the hearing organ. Halperin considers that this can be related to the small possibility of the botulinic toxin paralyzing the sensitory organs. These conclusions do not appear to be surficiently corroborated, in our ppinion.

3. Paralysis of the Nervous System during Botulinic Intoxination

Paralysis of the nervous system, especially the central one, in botulism is expressed so sharply that for a long time clinical workers concentrated all of their attention on it. Only during the past 25 years, Soviet doctors have discovered also other serious paralyses in botulism patients. As a result of the action by toxin

upon the central nervous system, one notices at the beginning of the disease restlessness, fear, poor slumber, in certain cases hysterical attacks. In the end stages, as has been indicated, apathy and sleepiness is observed. In connection with paralysis of the nuclei in the medulla oblongata of botulism patients, a clear picture of bulbar syndrome. Disturbance of the deglutition, aphonia, unbalanced breathing and heart activity are observed. When the intoxication becomes more severe, the patients complain of difficulty in breathing which develops in connection with paralysis of the respiratory channels as well as in connection with the partial or complete paralysis of the diaphragm. Appearances of asphyxiation progress, and there usually takes place a progressive collapse due to asphyxiation which ends in death.

Mirtovski and Govseev (1937) studied the condition of the nervous system in botulism patients. They observed 75 sick persons - 39 men and 36 women. The authors investigated the disease in various periods of its development.

The first symptoms, indicating a paralysis of the nervous system in botulism patients, appear to be the eye symptoms. They almost always bring the sick person to the doctor. Most often, the patient complains that he "sees double" and about the rapid fatigue of vision. Next a weakening of the peripheral musculature is noticable, the patient complains about the difficulty in movement, rapid weariness. During further action by the toxin upon the nervous system, the weakness of movement quickly increases. At certain times, a muscular weakness in

in the legs and hands is observed. As a result of disturbances to the muscular functions, the patient is unable to hold up his head and it falls either forward or to one side. The sensory spheres in the sick persons undergo almost no change. All appearances of sensitivity are preserved.

Further, the eye symptoms progress. The internal and side eye muscles are stricken. Very often paralysis of the eye-lids (ptosis) develops. In certain cases, paralysis of the right and left eye-lid is expressed differently. Quite often paresis of the internal eye muscles and disturbance in accomodation are observed. Expansion of the eye-pupil and a weak reaction to color are noticable in all botulism patients. In separate cases, the toxin paralyzes the vision nerve.

Kazas, Kovarskaya, Krol, Warshavskaya and Berman (1937), while examining 80 botulism patients, established a new hitherto completely unknown symptom in botulism - the absence or sharp decrease in the reflex of the eye cornea. This symptom was manifested by 55 patients. Barbel (1932) in one case observed paralysis of the eye bottom as a result of the central nervous system having been stricken by the toxin of botulism.

Death from botulism occurs after paralysis of breathing during continuation of the activity by the heart. The sick persons always die with a clear consciousness; even in the period of agony, loss of consciousness is noted very rarely.

4. Paralysis of the Heart-Vacular Systems

The cardiac-vascular systems suffer considerable changes in botulism. For a long time, the syndrome was completely disregarded. In the study of botulism epidemics the main attention, a. has been mentioned, was directed toward the paralysis of the nervous system. It was considered already known that the cardiac-vascular system undergoes small changes in botulism but that it is the most resistant. However a careful study of botulism patients and a pathologic-anatomical investigation showed that very strong paralyses of the heart and vessels may take place in botulism. Shteinberg (1937) notes the sharp disturbance in blood circulation of botulism patients which is expressed in "a decreased flow of blood toward the right part of the heart, in a decreased diastolic filling of the heart." These phenomena are caused not only by a serious disturbance to the innervation of the heart but also by a paralysis to the cardiac muscles by the botulinic toxin, which has been substantiated through pathologic-anatomical research by Kuraeva.

During the presence of these symptoms from the side of the heart, it is difficult to sufficiently explain the fulness and tenseness of the pulse. It is assumed that such a condition of the pulse in botulism patients depends upon the strong contraction of the peripheral blood vessels.

Shteinberg (1937), having studied the capillarioscopic pictures of 42 persons sick with botulism, discovered a contraction of the arterial node and a simultaneous expansion of the vein node.

The author explains this phenomenon only by expressing the hypothesis that, if the capillatioscopic tests were applied from the first day of the disease, then the spasm of vessels possibly would appear more accurately and would be met with more often. Katsnelson, Kutsygin and Kheifets (1937) studied 105 botulism patients for the condition of their blood pressure; they discovered in one-half of the sick persons higher as well as systolic and diastolic blood pressure, whereas in the remaining it was normal.

It was impossible to make these data agree with those for the heart paralysis, about which we spoke in the foregoing, if there had not been determined clinical data on the considerable degree of contraction by the blood vessels in botulism. This phenomenon is often substantiated through the observed dryness of the mucous glands and the paleness of the skin of botulism patients.

Clinical observations show that, apart from the contraction of blood vessels, the botulinic toxin causes difficulty in the entirety of the vessel wall, especially in the capillaries and the precapillaries. This was established by Katsnelson and Paperny (1937) who were studying the Rumpel-Leede phenomenon in botulism patients.

As is known, the essence of this phenomenonzlies in the compression of an extremity with an elastic bandage and the appearance below the place of compression of minute subcutaneous hemorrhages in the instance when the walls of the blood vessels have been affected by action of the intoxication. This phenomenon appeared to be positive in 34 of the 63 patients and was observed to be the most often met with in average or serious cases of botulism.

5. Changes in Temperature during Botulism

According to the data of most authors, a case of botulinic poisoning usually takes place with the temperature being normal.

Such a unanimity in views for a given case can be explained by the insufficient attention given to this problem. A fever in botulism was noted by Slutski, Govseev and Rosin (1937) who observed an epidemic of poisoning from sturgeon. In certain of the patients, the temperature remained higher than normal for a long time. Changli-Chaikin (1937) examined 40 victims of botulism; in 19 he noted a fever even before injecting the serum. The temperature was high also in 8 of the 34 persons sick with a serious form of botulism; in a group with average botulism, the temperature was high prior to the serum therapy in 15 out of 72 persons. In a large number of patients, the fever remained also after the serum therapy which can be explained by the action of the serum. In certain instances, the temperature remained high over a period of 9 to 14 days; in two cases - up to 24 days. Changli-Chaikin comes to the conclusion that a high temperature in botulism patients is connected with the viability of the botulism bacillus. Consequently, botulism in certain cases does not differ in its course from other infectious diseases.

6. Condition of Various Organs in Reconvalescents

In almost all works devoted to the study of clinical botulism, a very slow rate of reconvalescence is noted which at times lasts for months. This period is characterized by a gradual reestablishment

of all functions in organs disturbed by the action of the botulinic toxin. Very interesting material on this problem is given in the work of Rappoport, Lifshits and Varshavskaya (1937), who observed the condition of 46 reconvalescents for more than two months, after the latter had left the hospital. In all of these cases, the nervous system had been seriously stricken.

The special intensity of the process reestablishing the functions of organs was noticed for the first 15 days of reconvalescence. Among the symptoms, the first to pass was the damage to breathing and swallowing, considerably later - the head-ache, snuffles, aphonia, ptosis. Even later or simultaneously with these the general weakness, paralysis of the heart, miastenic phenomena, pain in muscles, the operation of the nerve stems were all relieved. In serious instances, return to health took place only after two to three months and at times even later. In one case a recidivity of acute snuffles was observed almost one month after reconvalescence.

Changli-Chaikin (1937) observed the condition of the cardiac-vascular systems in 23 reconvalescents over a period of four to six months. All of the patients, after leaving the hospital, complained about general weakness, rapid fatigue, and a strong heart beat during physical exertion. In persons who had suffered only a mild form of the disease, such a condition lasted from two to three weeks after release from the hospital; in those who had the serious form of botulism, substantially longer.

Reconvalescents, who underwent the serious and medium forms of botulism, complained often about pains in the heart area especially after physical exertion. In certain cases, an expansion of

the heart was ascertained radiologically. Changli-Chaikin considers that the clinical phenomena determinedly appear in the paralysis of the miocardia and in the vessels. In his conclusion, the author comes to the following: "It is necessary to conclude that the cardiac-vascular insufficiency, observed by us in botulinic reconvalescents, depended first of all upon the direct influence of the botulism toxin upon the cardiac muscle. This is supported by the clearly degenerative transformations in the miocardia, discovered by histological investigation. Similarly to the case during infectious miocardias of various etiologies (scarlet fever, dyptheria, typhus), the clinical picture characteristic for the miocardia paralysis appears after a more or less lengthy latent period."

In such a manner it was definitely established that paralysis of the cardiac-vascular systems in reconvalescents is observed just as often as during the acute period of the disease. At the end of the acute period of the illness, there develops a paralysis of the miocardia which also explains the presence among the reconvalescents of symptoms characteristic for an infectious miocardia condition.

Apart from the symptoms shown above, quite often miosis was observed in the reconvalescents. Paralysis of the skeleton muscles developed in later periods of the disease and were more often seen in those who had survived the heavy form of botulism. The pictures of the muscular illness, according to the data of Abramova, was very similar to the picture of acute muscular reumatism and was accompanied by pain and difficulty in movement. The muscles swelled

slightly, became very painful during contraction; fever was absent in this connection. Abramov observed 30 reconvalescents over a long period of time; they had overcome a serious or medium form of botulism; among these, 18 patients were stricken in the muscular system. After heat treatment, the miosis passed after one or two days; in the absence of treatment, the process lasted 7 to 10 days.

The above described symptoms in reconvalescents are very characteristic. On the one hand, they definitely show that the botulinic toxin causes a very deep pathologic transformation in a series of organs and tissues. Reestablishment of the stricken cells takes place very slowly; in this connection, some of the disturbances remain for life as is observed also in other infections and intoxications.

The course of the process of reconvalescence from botulism also gives rise to the hypothesis on the presence of a lengthy intoxication which may be connected with the multiplication of the microbe in the organs and the production of toxin. The picture of reconvalescence from botulism definitely indicates the toxic-infectious character of the disease.

IMMUNITY TO BOTULISM

Chapter VI

Immunity to botulism is a field on which very little study has been done. Up to the present time, we are unable to explain the various sensitivities toward the botulinic toxin in certain human beings and animals. In many cases of botulinic poisoning, when using the same quantity of poisonous food, certain members of a family died from botulism whereas others suffered a very serious form of botulism and still others had a light form of the disease or were not ill at all. Some authors explain this by the cluster-like location of the spores in the product. Similar instances were observed also in diseases caused by semi-fluid conserved products, and this explanation appeared to be unconfirmed. The hypothesis of Meier and Heiger (1921) that the insensitivity of certain persons to the botulinic toxin is explained by the presence of anti-toxin in their blood is also unsubstantiated. In this type of individual, in the majority of cases, anti-toxin was not discovered in the blood.

Individual insensitivity to the botulinic toxin is also met with among various animals. Certain laboratory animals - rabbits, guinea pigs and mice - manifest in experiments considerable resistance to the botulinic toxin. We were able to observe this several times. Minervin and Kotlyarevskaya (1937) set up a special test in order to explain the individual insensitivity of guinea pigs to the botulinic toxin. It appeared that of the 21 guinea pigs,

three had very little sensitivity to this poison. Insusceptibility to the botulinic toxin on the part of certain domestic animals, especially pigs and cattle, is also known.

Minervin and Kotlyarevskaya announced the hypothesis that resistance to the botulinic toxin is connected with the "insensitivity of tissues and cells, usually stricken during botulism, to the botulinic poison." As was shown in our previous experiments, this hypothesis is fully supported in the test with isolated organs. When infecting animals with the spores from the botulism stimulant, the cells and tissues of their organs may acquire immunity to the botulinic toxin.

In our research on the immunity to botulism, we set ourselves the task of explaining the separate aspects of the mechanism in this phenomenon.

1. The Anti-toxin Immunity of Cells in Organs

The possibility of obtaining an anti-toxin immunity to botulism has long been established. It was considered, in this connection, that the organism is protected from the intoxication exclusively by the anti-toxin found in its blood. The condition of the cells and organs was not taken into consideration.

Even VanErmeng attempted to create an anti-toxin immunity in animals by injecting small doses of botulinic toxin. The first attempts by Kempner (1897) to immunize rabbits and guinea pigs were unsuccessful. Forsman (1902) immunized these animals with a botulinic toxin heated during 30 minutes at 60 degrees, but he received

unsatisfactory results. Later Kempner was able to immunize goats to toxin with harmless methylbenzene, after which he applied the concentrated toxin. Utilizing this method for the immunization of goats, Forsman obtained a very strong serum.

Continuing along this line, botulinic serum of various activeness was obtained by a number of authors: Graham, Bruckner, and Pontius (1918) on sheep, goats and bulls; Meier, Hurwitz, and Taussig (1918) on dogs; Leichs (1912), Bengston (1924), and Hateh (1924) on horses. These authors used pure toxin or a mixture of toxin with anti-toxin for the immunization. They all write about the difficulty of immunizing animals with small doses of the toxin. Dixon and Hoyt (1920) were successful in immunizing goats with the toxin but only for several months, and they noted that the goats reacted differently to the toxin. During the immunization of four horses with toxin, which was introduced in small but always increasing doses, Wheeler (1923) lost three horses, and the immunization of the survivors lasted six months. All of this data indicates that during immunization with small doses of the botulinic toxin, a sensibilitation of the animal organism takes place, and as a result they die from botulism. It is interesting that none of the researchers paid any attention to this important fact. The sensibilitizing properties of the botulinic toxin, prior to the investigations of Minervin and Kotlyarevskaya (1936), were completely unknown.

Important changes in the methodology for preparing anti-botulinic serum were introduced by the observations of Wainberg and Hoy (1924, 1925). These authors proposed the immunization of

animals with anatoxin, prepared by adding to the botulinic toxin from 0.3 to 0.8 percent of formalin and keeping this for a period of 12 to 17 days at a temperature of 27 degrees in a thermostat. The formalinized toxin possesses good anti-genous properties and does not precipitate intoxication phenomena during immunization of animals. Small animals (rabbits, guinea pigs) can be immunized by means of injecting under the skin 0;5 to one milliliter of anatoxin every day or at intervals of four to five days. They can also be immunized by the method of a single injection subcutaneously of 20 milliliters of anatoxin. Horses carry through the immunization well; at first they were given doses of 20 to 30 milliliters of the anatoxin which were gradually increased so that the last one may be equal to 500 to 600 milliliters.

In the Soviet Union, the anti-botulinic serum according to the method of Wainberg and Hoy was first obtained at the Mechnikov Institute on horses and later on goats (Komkova, 1930). At the Central Institute for Epidemological Medicine, Zelevinskaya and Volkova (1935) prepared an excellent serum on horses. Later on, according to the Wainberg and Hoy method, anti-botulinic serums for medical use were being prepared in many lands. The majority of these contained not more than 3,000 to 4,000 AE in one milliliter.

Soviet microbiologists in 1940 attained fine results in the conduct of anti-botulinic immunization. Thus for example, Burova and Kats (1940) by means of a separation of strongly toxigenous variants from industrial strains, obtained the cultures of the botulism stimulant, providing a very strong toxin. Altogether

a total of 10,000 to 25,000 Dlm in one milliliter of toxin, obtained from these strains, contained 250,000 to 300,000 Dlm in one milliliter. Burova and Kats, utilizing for the immunization a toxin of that strength together with irritants (acids, chlorous calciums), obtained serums with a high anti-botulinic titer. In one horse, having 750 AE, the titer rose to 35,000 AE; in a second with 4,000 it climbed to 75,000; and in a third from 500 to 40,000 AE in one milliliter.

The medical anti-botulinic serums possess specific, typical properties. Usually monovalent serums of types A, B, C, D, and E are prepared; then by means of mixing, a polyvalent serum is achieved. Serum is prepared in large quantities against the toxin of Bac. Botulinus, types A and B, as those which are most prevalent in nature.

In their experiments with crossed titration of serums of types A and B, Legru and Dzheramek (1935) discovered that one and the same amount of serum type A neutralizes 100 lethal doses of toxin type B. This was established even earlier by Jensen (1926). In his tests, one Dlm of the toxin type B neutralized itself through the addition of a large quantity of serum type A. According to the data of Bronfenbrenner (1924), the toxin type A in the presence of serum type C increases its activity, kills animals more rapidly, which was corroborated by Jensen. Dzheramek, titrating serum type A, prepared by Ramon, explained that one milliliter can neutralized 75 million Dlm of toxin type B. The serum B in his experiments neutralized 5,000 Dlm of homologous toxin and 300 Dlm of toxin A. In the opinion of the author, this indicates an affinity between toxins A and B.

It appears to us that these properties of serums A and B can be explained not only by the affinity toward toxins but also by the adsorbtion properties of the serums. It is known that the serums with a high specific titer are able to neutralize small quantities of other antigenes. It is also necessary to take into account the purity of the strains, utilized for the preparation of typical serums. This is supported by the experiments of Mason and Robinson (1935), who did not obtain a cross neutralization when titrating serums A and B. Serum A would not neutralize even a minimum amount of toxin B and , conversely, toxin A would not neutralize serum B.

Anti-botulinic immunization may be realized by means of introducing the anatoxin by mouth. As far back as 1905, Chichkin showed the possibility of immunization against botulism through the mouth. Studying this problem, Wainberg and Hoy (1924, 1925) introduced into rabbits through the mouth up to 50 milliliters of anatoxin. Serum taken on the 20th day of immunization neutralized four lethal doses of toxin. The same results were obtained when rabbits were immunized over a period of 12 days, using one to five milliliters of anatoxin.

Melnik and Starobinets (1936) established that subcutaneous immunization of animals against botulism is attainable very easily. Passive immunization against botulism also does not present any difficulty.

At the beginning of treatment for botulism with serum, this method did not find general acceptance. Negative judgements were even made on this problem. A commission for the study of botulism

in the United States came to the conclusion that serum does not manifest any healing action. The same conclusion was arrived at by Burke, Elder and Pishel (1921). In the opinion of these authors the serum shows healing qualities only if introduced intravenously prior to the appearance of symptoms or at the very first appearance of the disease. In all remaining cases, no benefit from the serum allegedly appeared. During their tests on monkeys, Dek and Bud (1928) also obtained no effect after introducing the serum when the symptoms appeared, caused by the toxin of botulism.

The unsuccessful instances of serum therapy for botulism can be explained apparently by the late introduction of the serum - after considerable quantities of the toxin had already united firmly with the organism tissues. Apart from this, in these cases serum was probably not of the type as had precipitated the disease. In treatment, it is necessary to apply only polyvalent serums. During the present time in the Soviet Union treatment of botulism with a polyvalent serum is widely applied. According to the data of Petrov (1936), Kanevski and Shapiro (1937), Kolesnikov, Dorofeev and Mamikhin (1937), Khozhainova (1938), Morozova (1941), it provides satisfactory results. Applying the treatment with serum in 58 cases of the botulism disease in its serious form, Shteinberg, Stanishevskaya and Berman (1937) came to the conclusion that the serum gives good results when introduced early in large quantities. It is necessary to introduce the serum intravenously at first 5 to 10 milliliters; during two to three hours later, 50 to 100 milliliters. The patients should also receive injections of the serum into the muscles during the first days of the disease several times with 50 to 100 milliliters each time.

In recent times Legra, Dzheramek and Levadit (1943, 1944, 1946) published reports on the treatment of botulism patients by means of serum in France. The treatment began with immunization by an anatoxin; then serum was introduced. Such a method provided very good results, especially in cases having a long incubation period. Good results with serum therapy for botulism were also announced by Marsden (1942). In the opinion of Minervin and Kotlyarevskaya (1938), Minervin and Batrak (1938), Minervin and Morgunova (1939), in order to achieve successful treatment of botulism, it is necessary to utilize not only and anti-toxic serum but also an anti-bacterial one. Under experimental conditions the application of an anti-bacterial serum for the treatment of botulism, in the report by Minervin and his collaborators, provided good results.

On the basis of the foregoing survey of basic research on the anti-toxic immunity toward botulism, it is apparent that nobody from among the investigators paid attention to the problem of the condition in the organ cells during active and passive immunity to botulism. All of the work done was devoted to the study of the resistance by the organism in relation to the titer of the anti-bodies. In this connection, the experiments of Galanova and Kravchenko established that the "anti-toxic active immunity during dyptheria is conditioned not only by the presence of the anti-toxin in the organism but also by the presence of immunity in the cells, their areactiveness, reconstruction to an extreme measure in relationship to these substrata B. Dyptheriae which are found in its protoplasma, and possibly in connection with substances which are secreted by a microbe into the surroundings" (Galanova, 1936).

Our further investigation was devoted to the explanation of the possibility for development on the part of similar phenomena during botulinic immunity.

In the previous experiments, we already showed that during the infection of rabbits and guinea pigs with the spores of the botulism stimulant, their organs can be areactive toward the botulinic toxin. In order to explain the mechanism of this phenomenon we established experimental observations over the immunization of animals by the botulinic anatoxin.

Guinea pigs were immunized subcutaneously with the anatoxin three times during five days; using one, two and three milliliters each time. The anatoxin was prepared by the method of Wainberg and Hoy in a medium of bull meat. After the immunization of the animals, $>5<20$ AE of the anti-toxin was maintained in the blood. Between the 7th and the 29th day after application of the anatoxin, the guinea pigs were killed. We used the Kravkova-Pisemski method in attempting to observe the reaction of vessels from the rear half of their bodies to the toxin of the botulism bacillus. In order to set up the tests with the vessels, the toxin was prepared in boulion from rabbit meat in order to eliminate an anaphylactic reaction by the vessels when solutions of the toxin were passed through. Such a reaction could take place in a case of utilizing the toxin, prepared in a medium of bull meat. The experiments were undertaken with purified dry toxin, initially undergoing dialysis, as was done in the preceding experiments. Prior to allowing the toxin solutions to pass through, the vessels of the guinea pigs were carefully washed

with a Ringer-Lokk solution until the blood had been eliminated in full. The vessels of five guinea pigs were tested on the 7th to 9th day, another five animals on the 17th to 19th day, and the remaining four on the 28th or 29th day after immunization. In all instances, the vessels of immunized animals reacted to the toxin in a weaker manner than did the vessels of the control and normal guinea pigs. While allowing the toxin in a dilution of 1:50,000; 1:25,000; 1:10,000 to pass through the vessels of the animals that were treated with anatoxin, these vessels provided an average contraction of 10, 12.1 and 13.2 percent. On the 28th and 29th day after immunization, irrespective of the decline in the titer of antitoxin in the blood to $>0.5<2$ AE, the vessels manifested a very weak reaction to the toxin at all times.

With the objective of establishing the cause for the small reaction to toxin by the vessels of immunized guinea pigs, a second series of tests was set up on six guinea pigs. The weak reaction could have depended upon the contraction of the vessels under the influence of the anatoxin and also upon the immune condition of the cells in the smooth musculature of the vessels. The guinea pigs were killed on the 15th to the 18th day after immunization by an anatoxin and experimental reaction of their vessels to adrenalin. If the areactive condition of the vessels to the toxin were nonspecific, then the vessles of the immunized animals would provide a weaker contraction to adrebalin than would the vessels of normal animals. However, as can be seen on Table 17, the vessels of guinea pigs immunized by anatoxin and the vessels of control animals not undergoing immunization, gave identical reactions of contraction to adrenalin.

Table 17. The Reaction to Adrenalin of Cells in the Smooth Musculature in Vessels of Guinea Pigs, Immunized with Anatoxin of Bac. Botulinus

Number in sequence	Date and dose at immunization			Titer of antitoxin in serum of (AE)	Date of test according to Kravkova method	Reaction of vessels to adrenalin				
	3.15.	3.21.	3.27.			Ringer-Lokk solution	1/1,000,000	Ringer-Lokk solution	1/500,000	Ringer-Lokk solution
1	1.0	2.0	3.0	> 5 < 10	4.12.	116	82	112	76	106
2	1.0	2.0	3.0	> 5 < 10	4.12.	120	86	116	70	116
3	1.0	2.0	3.0	> 5 < 10	4.15.	146	102	140	94	132
4	1.0	2.0	3.0	> 5 < 10	4.15..	140	70	138	68	124
5	1.0	2.0	3.0	> 5 < 10	4.13.	140	108	132	102	118
6	1.0	2.0	3.0	> 5 < 10	4.13.	140	98	134	96	120
Totals:						802	546	772	506	716
Average contraction of vessels							31.9%		34.5%	
Control Guinea Pigs										
1					4.11.	126	72	124	68	120
2					4.15.	100	60	98	58	96
3					4.13.	140	74	132	102	130
Totals:						366	206	354	228	346
Average contraction of vessels							43.7%		35.6%	

On the basis of these tests, it can be stated with assurance that a weaker reaction to the toxin by the vessels of guinea pigs, immunized by anatoxin, was dependent upon the immunity of the cells in the smooth musculature of the vessels to the botulinic toxin. Subsequently, the areactive consition of the vessels was specific.

In connection with the presence of anti-toxin in the blood of immunized animals, the hypothesis also arose that the weak reaction of their vessels may be connected with the neutralization of the toxin.

After washing the vessels with a Ringer-Lokk solution, there still could have remained blood containing toxin, which neutralizing the toxin could have simulated a weak reaction of the vessel cells to the toxin. Apart from this, the anti-bodies found on the surface of the vessel cells in the form of so-called sessile receptors could protect the cells from the action of the toxin upon them. It was necessary to explain whether the areactive condition of the vessels depended upon the immunological consition of the cell protoplasma or upon the anti-toxin found in the blood.

In the third series of tests, the guinea pigs received subcutaneous injections of 10 milliliters of anti-botulinic serum which contained 500 AE in one milliliter; subsequently during 24 to 48 hours, the reaction of their vessels to the botulinic toxin was observed. Prior to passing the toxin solution through the vessels of these animals, the latter were washed as carefully as had been done in the preceding experiments. A total of nine tests were set up (Table 18); the vessels of the passively immunized guinea pigs

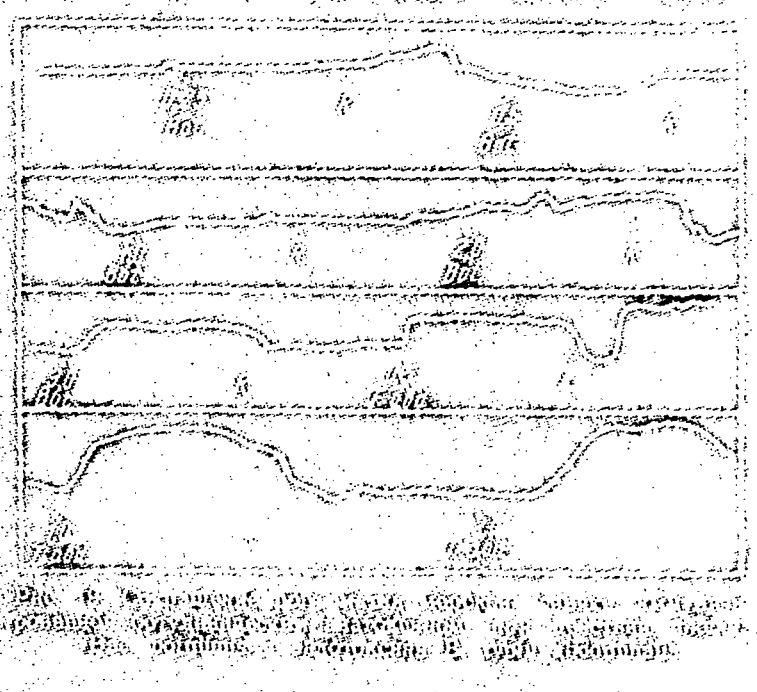
Table 18. The Absence of a Passive Transmittal of Immunity by Cells in the Smooth Musculature in the Vessels of Guinea Pigs during Introduction of Anti-Botulinic Serum

Number in sequence	Number of guinea pig	Quantity of serum injected (in milliliters)	Date of serum injection	Date of test on Kravkova apparatus	Reaction of vessels to toxin						
					Ringer-Lokk solution	1:50,000	Ringer-Lokk solution	1:25,000	Ringer-Lokk solution	1:10,000	Ringer-Lokk solution
1	1	10	1.11.	1.13.	138	120	130	66	84	30	40
2	2	10	1.11.	1.13.	110	90	80	64	82	40	66
3	3	10	1.11.	1.13.	124	114	120	100	110	50	70
4	4	10	1.15.	1.17.	80	56	64	40	56	40	50
5	7	10	1.20.	1.21.	128	94	100	66	90	45	60
6	8	10	1.20.	1.21.	100	78	98	50	68	30	52
7	9	10	1.28.	1.29.	130	112	114	100	104	70	80
8	10	10	1.28.	1.29.	100	88	100	60	70	44	50
9	11	10	1.28.	1.29.	110	90	110	84	104	40	50
			Totals		1,020	842	916	630	768	389	518
			Average contraction of vessels			17.5%		31.3%		49.4%	
				Control Guinea Pigs							
1				1.17.	130	120	128	70	118	50	65
2				1.19.	90	60	78	64	74	34	45
3				1.21.	90	76	90	68	72	50	62
4				1.29.	90	60	90	84	90	36	50
5				1.30.	120	100	116	70	110	60	72
			Totals		520	416	502	356	464	230	294
			Average contraction of vessels			20.0%		29.1%		50.4%	

Figure 18. Contraction of Beak on Uterus in Guinea Pig, Immunized with a Botulinic Anatoxin, during the Action by Toxin of Bac. Botulinus and the Endotoxin of B. Typhi Abdominalis.

gave the same reaction to the toxin as the vessels of normal animals. It is obvious from these tests that the areactive consition of the vessels in guinea pigs, immunized by anatoxin, does not depend upon anti-bodies but is connected with the deep immunological transformations of the protoplasma itself in the cells of the smooth musculature of vessels.

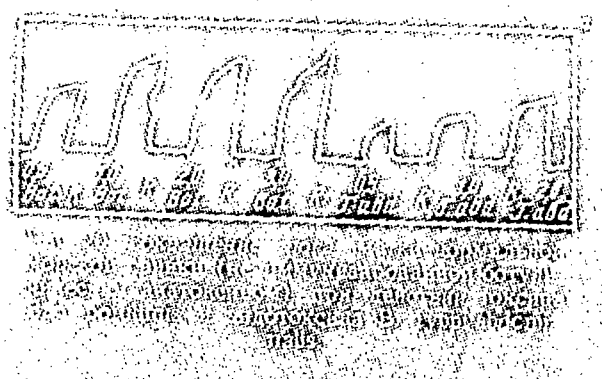
Figure 19. Contraction of the Intestine in a Guinea Pig, Immunized with a Botulinic Anatoxin, during the Action by Toxin of Bac. Botulinus and the Endotoxin of B. Typhi Abdominalis.



The described tests indicate the impossibility of passive transmittal of immunity by cells with a serum containing anti-toxin. Consequently, the relationship was experimentally established between the cellular anti-toxic immunity and the antibodies. Hence, the unlastingness and instability of the anti-toxic humoral immunity becomes understandable; it arises as a result of introducing the serum with ready anti-bodies into the organism.

The fourth series of experiments was arranged in order to study the immunity of cells of the smooth musculature in the uterus and intestines of guinea pigs, immunized with the anatoxin from the botulism bacillus. The condition of these organs was observed in those guinea pigs which had already been studied for the reaction of vessels to toxin after immunization. The tests were set up simultaneously with the vessels according to the Kravkova-Pisemski method, and with the uterus and intestines in the Schultz-Delia apparatus. In order to cause an anaphylactic reaction by these

Figure 20. Contraction of the Uterus Beak in a Normal Guinea Pig (not Immunized with the Botulinic Anatoxin) during the Action of the Toxin Bac. Botulinus and the Endotoxin B. Typhi Abdominalis.



organs after immunization with the anatoxin, the experiments utilized botulinic toxin prepared in a boullion from rabbit meat. The dry toxin was diluted 1:100 and submitted to dialysis.

Altogether 12 tests were conducted in the Schultz-Delia apparatus with the uterus and intestine. In the five guinea pigs, the reaction of organs was studied on the 7th to 9th day; in five guinea pigs on the 17th to 19th day; and in two of the animals on the 29th day after immunization by the anatoxin. When comparing Figures 18 and 19 (reaction of organs in an immunized guinea pig) with Figures 20 and 21 (reaction of organs in a normal guinea pig), we see that the reaction of the uterus and intestine to the botulinic toxin in these animals was different. The organs of the immunized guinea pig did not at all strengthen their contraction in the presence of the toxin, at the same time that the organs of a normal animal provided a clearly expressed reaction to the botulinic poison.

On Figures 22 and 23, there is protrayed the average height of curves for the contraction of the organs (uterus and intestine) in guinea pigs that have been thrice immunized with anatoxin. We see that the reaction by the organs of these animals takes place only on the last dose of the toxin. On Figures 24 and 25, there is portrayed the average reaction to the toxin by organs in normal animals: considerable strengthening of the contraction in the presence of the botulinic toxin. It is necessary to note that the contraction by immunized organs and normal animals in the presence of the endotoxin of the typhoid-fever stimulant was identical. This testifies to the fact that an areactive condition of organs in immunized

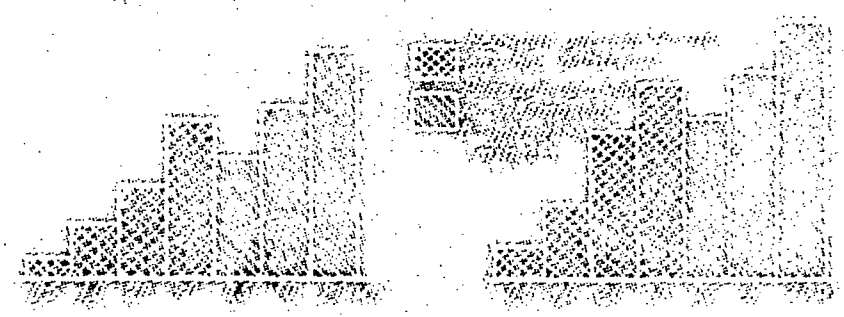
Figure 21. Contraction of the Intestine in a Normal Guinea Pig (not Immunized with the Botulinic Anatoxin) during the Action of the Toxin Bac. Botulinus and the Endotoxin of B. Typhi Abdominalis.

Reaction of Organs to Toxin of Bac. Botulinus

Reaction of Organs to Endotoxin of B.Typhi Abdominalis

Figure 22. Average Height of Curves for Contraction of Uteruses in Guinea Pigs, Immunized by Anatoxin of Bac. Botulinus.

Figure 23. Average Height of Curves for Contraction of Intestines in Guinea Pigs, Immunized by Anatoxin of Bac. Botulinus.



guinea pigs was specific. The cells of the smooth musculature in the uterus and intestines of these animals achieved immunity from the poison of botulism: contraction of the organs in immunized guinea pigs in the presence of botulinic toxin was not increased.

It can be concluded from the described experiments as follows: In the immunization of animals with a botulinic anatoxin, there occurs not only a collection of anti-bodies in the blood but also immunological transformations in the cells of the smooth musculature of the vessels in the uterus and intestines. The organs of the immunized animals become areactive to the botulinic poison.

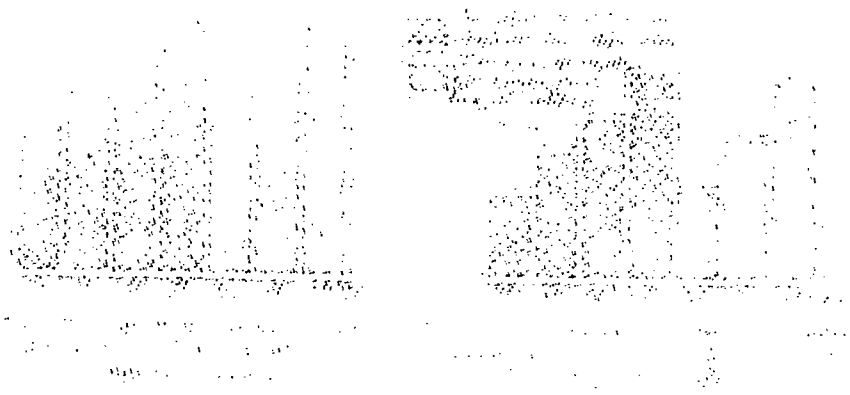
Reaction of organs to toxin of Bac.Botulinus

Reaction of organs to endotoxin of B.Typhi Abdominalis

Figure 24. Average height of curves for contradtion of uterus in normal guinea pigs.

Figure 25. Average height of curves for contraction of intestines in normal guinea pigs.

The portrayed data are very important for an explanation of the mechanism of immunity in these organs, which immunity occurs in animals as a result of a lengthy symptomless botulinic infection. In the immunization of guinea pigs with a preparation made from the



botulinic toxin - anatoxin, the cells of their organs acquire immunity. Such a condition takes place under the influence of the botulinic infection, caused by the spores from the bacillus of botulism. This supports our conclusion to the effect that during the symptomless botulinic infection toxin is formed, which toxin creates an immune reconstruction of the cells in the smooth musculature of the organs. In connection with the ability of the cells in the organism to acquire areactiveness toward the botulinic toxin after immunization by anatoxin, the hypothesis of Legra and Dzheramek (1943) about utilizing it for the treatment of botulism should be taken into consideration. According to the data of the authors, this method of treatment provided very good results, especially in cases with a long incubation period. It is completely clear that the anatoxin must be applied in all instances where botulism is suspected and also as a prophylactic means for persons consuming products contaminated by the botulism microbe.

Numerous experiments have shown that in the immunization of animals against brucellosis, typhoid fever, and also during their infection by the stimulants of these diseases, the cells in the organs of animals (vessels, uteruses, intestines) acquired immunity to the endotoxin of these microbes. Apart from this, agglutinates were produced in considerable quantities in the blood. Kvarchenko in his research conducted on the stimulant of typhoid fever showed that during the disappearance of agglutinates, the cellular areactiveness toward the endo-toxin is preserved.

We established the absence of any relationship between the immunity of cells toward B. abortus and the presence of anti-bodies in the blood as well as the impossibility of passive transmittal of immunity and allergy by the cells in this infection (1940).

The possibility of obtaining immunity in the cells of the smooth musculature of the uterus and intestine toward poisonous products of the dyptheria stimulant was established by Galanova and Kravchenko. We together with Bulatova conducted our research according to the Kravkova-Bisemski method on the vessels of kidneys immunized by anatoxin of horses - producers of medical serums. In the experiments, we observed the reaction of kidney vessels in 15 horses, immunized by a dyptheria anatoxin; 12 horses, immunized with a tetanus anatoxin; and 14 normal horses. A considerably weaker reaction was obtained to the tetanus and dyptheria toxin in the cells of the smooth muscles of kidneys in immunized horses than the reaction to these poisons by the cells in the vessels of kidneys in normal horses. The areactiveness to the toxin by kidney vessels in horses, immunized by anatoxin, did not depend upon the titer of anti-toxin in the blood of these horses. Such results were obtained during tests on rabbits and guinea pigs.

Subsequently, in our research on the cellular anti-toxic immunity toward botulism and also to tetanus and dyptheria, we established such a rule in obtaining it as is true of the anti-bacterial immunity during brucellosis and typhoid fever. During the immunization with bacteria and the products of their activity - by means of the toxins there occurs not only a collection of anti-bodies but also a transformation in the reactiveness toward the anti-genes of cells in the animals organs. The areactive condition

of the cells in this and in other cases depends upon the deep immunological transformations of their protoplasma under the influence of the anti-gene. It is necessary to keep in mind that the immunological transformations do not take place in all cells simultaneously and to the same degree. The differentiation of the immune reconstruction in various cells was established by the work of Kravchenko (1941).

2. Changes in the Reactiveness of the Central Nervous System during Immunization against Botulism

In botulism, the earliest symptoms of the disease arise as a result of paralysis in the central nervous system caused by the toxin. For this reason the problem of guarding the central nervous system during serum therapy possesses a very large practical significance. It is known that the serum shows a good effect in the treatment of botulism only during an early injection into the ill person. Later application of serum therapy provides considerably poorer results.

Less effective in the medical action of the serum, during a late injection into a patient, was explained in that the anti-toxin did not penetrate through the hemato-encephalic barrier into the central nervous system and that the toxin located there did not undergo neutralization. In this connection, paralysis of the central nervous system and of other organs always increases, and the patient dies from botulism.

The majority of researchers conducted a study of the penetrability of the hemato-encephalic barrier in relation to the agglutinates,

hemolysins and precipitins. Such a direction of work was related to the lighter and simpler methods of discovering these anti-bodies in the blood and in the spinal cord fluid of animals. On the basis of results obtained with the agglutinins, hemolysins and precipitins it was assumed that also the anti-toxins penetrate into the spinal cord fluid during their circulation in the blood of immunized animals.

However, these observations although containing data on the absence of anti-toxins in the spinal cord fluid, were all not absolutely confirmable in connection with the fact that the experiments were conducted at a low titer of anti-toxins in the blood of the animals.

All of this served as a reason for launching together with Bulatova in 1947 research for the purpose of explaining the possibility of penetration by the botulinic anti-toxin into the spinal cord fluid during a large content of it in the blood of actively and passively immunized animals. These observations were of interest, because in the serum therapy for botulism and tetanus usually large quantities of anti-toxic serum are injected, and during which the titer of the anti-toxin in the blood of patients is very high.

Tests were conducted on rabbits with the botulinic anti-toxin. At first we observed the blood and liquiform in the animals for the presence of anti-toxin. After this, the rabbits were given intravenously various amounts of anti-botulinic serum (from 3 to 26 milliliters, 2200 - 20000 AE); then during four hours the liquiform

and the blood was observed. In this connection, it was found that in all 20 rabbits anti-toxin was absent from the liquiform at the same time that there was 15 to 30 AE in the blood.

Similar experiments were set up on 13 rabbits, subcutaneously immunized by anatoxin with 3, 5 and 10 milliliters at intervals of 5 to 6 days. On the 8th to 11th twenty-four hour period after the immunization, the titer of anti-toxin in the blood and liquiform were determined. The anti-toxin was absent from the liquiform, and in the blood it was found from 5 to 50 AE.

Consequently during a passive and active immunization of rabbits against botulism, regardless of the high titer of anti-toxin in the blood, it does not penetrate into the spinal cord fluid.

In order to study this important problem, we conducted more detailed observations of rabbits and colts into which were injected large quantities of heterogenious anti-tetanus serum. The experiment included 42 rabbits and one colt (Matveev, Kassil and Sokolov, 1946). First of all, in order to determine the anti-toxin in the standard, blood and liquiform was taken from the rabbits. The titer of the anti-toxin was below 0.001 AE and only in two cases was it equal to 0.001 AE in one milliliter. After this the animals received intravenously injections of the anti-tetanus serum: three rabbits, 50 milliliters; four rabbits, 70 to 75 milliliters; and eight rabbits, 70 to 90 milliliters. The serum contained 400 AE in one milliliter. In such a manner, each rabbit received from 20,000 to 40,000 AE, i.e. from 10,000 to 20,000 AE per one kilogramme of weight. Computing this on the basis of the weight of

the average human being (60 kilogrammes), this would amount to between 60,000 and 1,200,000 AE (up to three liters of serum).

In order to eliminate the reaction, occuring during the injection of large quantities of the serum, the latter was introduced slowly with 20 to 25 milliliters at a time and intervals every 10 to 15 minutes. Usually, besides a small asthma, no other phenomena are observed in the animals.

During 30 minutes after introduction of the serum in rabbits, blood and liquiform was taken for determination of anti-toxin. In eight cases out of 15, an insignificant increase in the amount of anti-toxin in the liquiform was noted - up to 0.01 AE in one milliliter during the presence in the blood of 100 AE in three rabbits; in four rabbits, 150 AE; and in eight rabbits, 200 AE in one milliliter.

Corelation of the liquiform: the blood comprised 1:10,000; 1:15,000; 1:20,000. The titer of the anti-toxin in rabbits was checked during one, two, three and four hours; it usually remained without any change. During 24 hours the titer of the anti-toxin in the liquiform of rabbits from the last group increased to 0.1 AE, whereas in the blood it dropped to 180 AE (Figure 26).

After 24 hours, a rapid drop in the anti-toxin titer took place. During the course of three 24-hour periods, its titer in the liquiform again decreased to 0.01 AE and in the blood to 90 AE. Up to the eighth day, the quantity of anti-toxin in the liquiform remained unchanged; whereas in the blood, it kept dropping in all of this time. On the 17th day, the quantity of anti-toxin in the liquiform and in the blood had returned to normal.

Colt no. 19 weighing 180 kilogrammes, in which normally there was less than 0.001 AE in the liquiform and blood, received 1470 milliliters of bull serum with 600 AE in one milliliter (882,000 AE). At the time the serum was introduced, a slight panting was noticed which continued for not longer than 10 to 15 minutes. The experiment represented considerable importance because at first the anti-botulinic bull serum in a large quantity for an intravenous injection on a large animal; this was a pioneering test. In this connection, it was very important that not toxic phenomena were observed in the colt.

We had made the hypothesis that during an intravenous introduction into the colt of large quantities of bull, i.e. heterogenous anti-tetanus serum, the tetanus anti-bodies would penetrate more easily into the liquiform through the hemato-encephalic barrier than after injection of the horse serum.

This hypothesis was not supported by the test. During two hours after introduction of the serum into the little colt, the titer of anti-bodies in No. 19 in the liquiform rose to 0.01 AE; after 24 hours, to 0.1 AE; after which on the seventh day, it dropped to 0.01 AE in one milliliter.

Remaining on this level to the 17th day, the anti-toxin titer in the liquiform began to decrease gradually and returned to normal on the 25th day after injection of the serum. In the blood of the colt after two hours from the time of serum injection, there was 30 AE; on the fourth day, the titer began to drop gradually; and on the 36th day, it attained its normal condition (Figure 27).

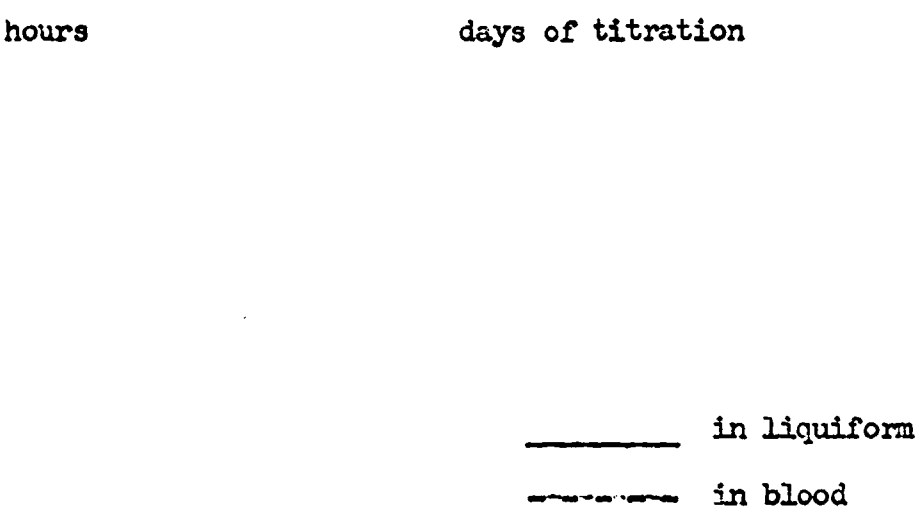
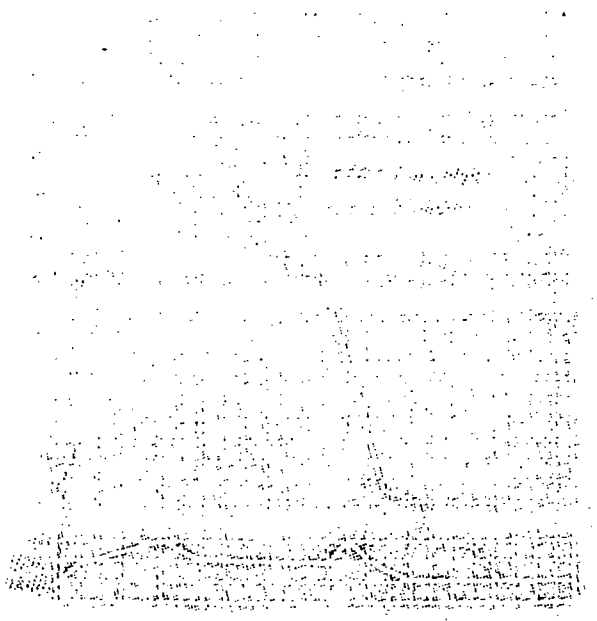


Figure 26. Changes in the Anti-toxin Titer in the Liquiform and Blood of Rabbits during Introduction of Horse Serum

These experiments show that in rabbits and colts, the hemato-encephalic barrier durimg intravenous introduction of large quantities of heterogenous serum is practically inpenetrable for the tetanus anti-toxin. Regardless of the high titer of the anti-toxin in the blood (for the colt - 30 AE; for the rabbits - up to 200 AE in one milliliter), the anti-body titer in liquiform increased only insignificantly from 0.01 to 0.1 AE in one milliliter.

In this connection, it was observed in rabbits that the anti-toxin titer dropped very rapidly in the blood. Massive doses of the serum, equal to 1/20 of the rabbit's weight, provided a passive immunity lasting bot more than 15 to 16 days. At the same time,



the passive immunity in the colt continued until the 26th day after injection of the serum.

The following series of experiments was set up on rabbits and on a colt by injecting homologous anti-tetanus serums into them (for the rabbits, a rabbit serum; for the colt, a horse serum).

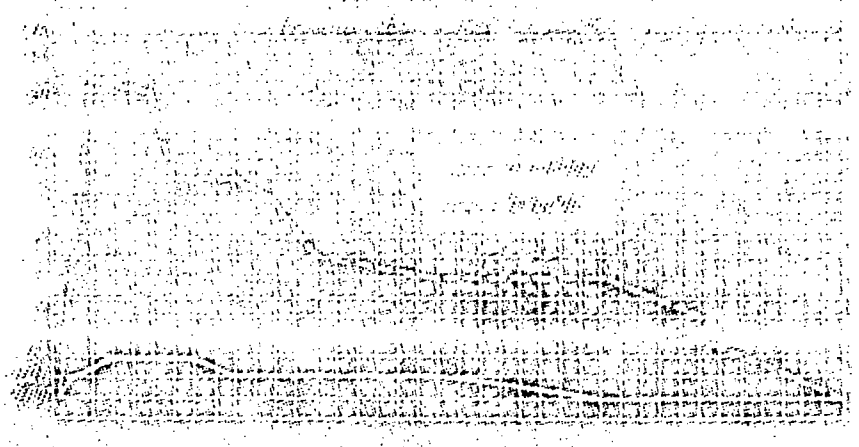
Hours — Days of titration

———— in liquiform

- - - - - in blood

Figure 27. Changes of the Anti-toxin Titer in Liquiform and Blood of Colt no. 19 during Introduction of a Bull Serum.

In order to obtain an anti-toxin serum, the rabbits were immunized subcutaneously with a tetanus anatoxin. Then the animals were uncovered and their serum introduced intravenously into fresh rabbits. Three rabbits, possessing an average of 0.001 AE in the liquiform and in the blood, received: the first, 30 milliliters - a total of 400 AE; the second, 33 milliliters - 1,000 AE; and the third, 40 milliliters - 800 AE. In the blood of these rabbits over a period of two days, there was an average of seven AE in one milliliter.



After this the anti-toxin titer began to fall rapidly, and on the 10th day it almost reached normal. During two hours after the injection of anti-toxin serum, the anti-body titer in the liquiform of rabbits was equal to 0.01 AE. On this level, it was maintained for 48 hours; then, after five days, it decreased to normal.

An analogous test was set up on colt no. 1 with a weight of 112 kilogrammes, in which the norm was 0.001 AE in the liquiform and in the blood. He received 1,400 milliliters intravenously and subcutaneously another 120 milliliters of anti-tetanus horse serum, totalling 1,770,000 AE. In colt no. 1, a considerable tension of the passive immunity was achieved. During two hours, the blood contained 100 AE; after this, the anti-toxin titer in the blood began to decrease. After 48 hours, it had dropped to 70 AE. It stayed on that level until the eighth day, then dropped to 40 AE (in the course of two days), after which it again increased over four days to 60 AE. From that moment, a slow decrease in the titer commenced; this continued over a period of 102 days. The extraordinary length of the passive immunity in this colt - almost 3½ months - attracts attention. We assume that this was dependent upon the introduction of the homologous serum. After two hours there was up to 0.5 AE in the liquiform, on the second day the titer had increased to 1 AE, qnd on the fourth day it again dropped to 0.5 AE. Maintaining itself on this level until the eighth day, the anti-toxin titer in the liquiform dropped on the 14th day to 0.1 AE and remained like that for a period of 45 days. Only after 70 days did the anti-toxin titer in the liquiform return to normal (Figure 28).

Hours Days of titration

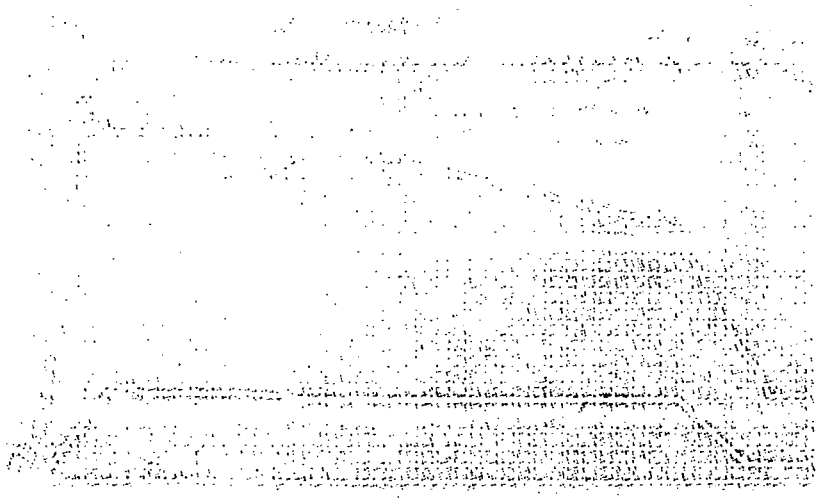
——— in liquiform

- - - - in blood

Figure 28. Changes in the Anti-toxin Titer in Liquiform and in Blood of a Colt during Introduction of Horse Serum

In this series, we obtained different results (at first glance) from those achieved in the tests on rabbits and the colt. During the intravenous injection of homologous anti-toxic serum into the rabbits, penetration by anti-bodies into the liquiform was not observed. It is possible that this was connected with the comparatively small tension in their passive immunity. The colt showed a different picture. It was noticed that he had a small increase in the anti-body titer in the liquiform during the introduction of a large quantity of homologous anti-toxin serum (up to 1 AE).

The comparatively high level of anti-bodies in the liquiform of colt no. 1, we explain in part by the blood falling into the liquiform at the time of puncturing and in part by the lowered resistance of the barrier in the young specimen. When there was a high content of anti-bodies in the blood, the presence of 0.001 to 0.01 milliliters of blood in the observed portion of liquiform



was sufficient to increase the titer of anti-bodies up to an average of between 0.5 and 1 AE. In view of the fact that the puncturing was conducted often, it was impossible to completely eliminate the penetration of blood into the liquiform. We are taking for granted that a small admixture of blood apparently caused certain increases of the titer in the liquiform.

Considering that the colt received a massive dose of serum and that the resistance barrier in young animals is considerably lower than in full-grown ones, it follows to agree that even when injecting large quantities of homologous serum the hemato-encephalic barrier allows only small quantities of tetanus anti-bodies into the liquiform.

On the basis of conducted experiments, we can come to the conclusion that during intravenous injections of large quantities of heterogenous or homologous anti-toxin serums into healthy animals it is possible to discover very insignificant quantities of anti-toxin at the time that the blood of animals may contain it in large quantities.

Consequently, during the serum therapy of botulism and tetanus the anti-toxin in practice does not penetrate into the reservoir of liquiform. However this still does not prove the defenselessness of the central nervous system during the serum therapy for these infections.

As has been shown in the foregoing, the absence of a medical effect on botulism when the serum is injected late was explained by claiming that the anti-toxin does not penetrate into the central nervous system and that the toxin already there remained unbound.

In order to check these opinions, together with Bulatova we launched upon a study of the condition on the central nervous system during active and passive immunization against botulism.

The condition of the central nervous system during the immunization against toxic infections remains to this day unexplained.

Ru and Borrel (1898) and also Ponomarev (1935) on the basis of their research came to the conclusion that the central nervous system during active and passive immunization against tetanus and dyptheria remains defenseless.

The experiments of Van den Hoven (1933) and also of Decombe (1929) conducted on a small number of animals appear to be unconfirmed, although the authors came to the conclusion about the defensiveness of the central nervous system during immunization against dyptheria and tetanus. The basic insufficiency of these experiments is involved in the fact that the animals (rabbits and guinea pigs) after immunization received the toxin through the cerebrum. In such a method of introducing the toxin, it is necessary to wound the capillaries in the brain tissue. Blood is released, and this neutralizes the toxin, so the animals remain healthy. For this reason, the experiments of Van den Hoven and Decombe appear to be unprovable.

Ascertainable data on the defensiveness of the central nervous system, after immunization against cerebro-spinal epidemic meningitis, were undertaken and announced by Zdrodovski and Golinevich (1934) as well as by Trotski, Sviridova and Ginzburg (1935).

In the course of our research, conducted together with Sokolov (1947), it was established that the absence of the tenatus anti-toxin in the reservoir of liquiform does not appear to be proof of the defenselessness of the nervous system against tetanus toxin. During the absence of the anti-toxin from the liquiform of rabbits, the central nervous system was found to be areactive to large doses of toxin suboccipitally injected into the animals.

We have set ourselves the goal to explain (Bulatova and Matveev, 1949) whether the central nervous system in rabbits is defended during passive and active immunization against botulism as well as to study the mechanism of this phenomenon taking place during immunization against botulism and tetanus.

The first series of experiments was established with the passive immunization of rabbits, into which anti-botulinic serum in various quantities was injected intravenously. Prior to the serum injection, the anti-toxin in the liquiform and blood of the animals was absent; usually, there was less than 1/1000 AE in one milliliter.

In the first test the rabbits received 26 milliliters (20,000 AE) of the serum, after a suboccipital injection of up to 20 Dlm of the botulinic toxin, and all remained healthy. The toxin was introduced during four hours after injection of the serum.

Then we conducted 10 other experiments in which the rabbits received injections intravenously of 10; 5; 3; 1.5; 1; 0.5 milliliters of anti-botulinic serum (corresponding to 7500, 3750, 2200, 1100, 750, 375 AE). The liquiform contained, during four hours after the intravenous injection of serum, 1/1000 AE; the blood had

between 5 and 30 AE.

Of the 46 rabbits that received suboccipitally 200 Dlm of the botulinic toxin each during four hours after the serum injection, five died from botulism, three from other causes, and the remaining 29 rabbits remained healthy without any observable symptoms of the disease.

In the course of subsequent tests, all three rabbits received 0.25 milliliter of serum (190 AE), then in a suboccipital injection during four hours 200 Dlm of the toxin; all died from botulism. This quantity of serum did not defend the central nervous system of the animals from 200 Dlm of the botulinic toxin. When increasing the quantity of toxin up to between 400 and 800 Dlm, the injection of even 10 ml milliliters of serum (7,500 AE) could not preserve the central nervous system of rabbits from the botulinic toxin (Table 19).

It is obvious from the described experiments that during the suboccipital injection of large botulinic toxin doses, small quantities of serum injected intravenously will maintain the central nervous system. An important fact in this connection appears to be the absence of any increase in the quantity of anti-toxin in the reservoir of liquiform.

A second series of tests was set up with actively immunized rabbits. Prior to their immunization, a titer of the antitoxin in the liquiform and blood was conducted on them. This process indicated less than 1/1000 AE in one milliliter. After this the rabbits received subcutaneous injections of botulinic anatoxin in amounts of 3; 5; 9; 10 milliliters at intervals of

Table 19. Changes in the Reactiveness of the Central Nervous Systems of Rabbits during a Passive Immunization against Botulism. Suboccipital Injection of Toxin.

Experiment											Control			
Number of test	Number of rabbits	Intravenous injection of serum (in milliliters)	Quantity of AE	Number of hours during which toxin was injected	Quantity of AE before injection of toxin		Suboccipital injection of Dlm-toxin	Number of rabbits after injection toxin			number of rabbits in control	Quantity of AE before injection of toxin		Suboccipital injection Dlm	Condition of rabbit
					in liquiform	in blood		remained healthy	died from botulism	died from other		in liquiform	in blood		
1	8	26	20,000	4	---- ----	----	200	2	--	-	-	------	------	-	-
2	5	10	7,500	4	<1/100	>20<30	200	4	--	1	1	<1/1000	<1/1000	1	Died from botulism on 4th day
3	3	5	3,750	4	≤1/1000	>20	200	2	1	-	-	-	-	-	-
4	5	3	2,200	4	≤1/1000	≥15≤25	200	4	-	1	-	-	-	-	-
5	3	3	2,200	4	<1/1000	≥20<30	200	2	1	-	1	<1/1000	<1/1000	1	Died from botulism on 4th day
6	4	1.5	1,100	4	≤1/1000	=20	200	3	1	-	-	-	-	-	-
7	5	1.5	1,100	4	<1/1000	=20	200	4	-	1	1	<1/1000	<1/1000	1	Died from botulism on 4th day
8	2	1.0	750	4	<1/1000	>10<15	200	1	1	-	-	-	-	-	-
9	3	1.0	750	4	<1/1000	=15	200	2	1	-	1	<1/1000	<1/1000	1	Died from botulism on 4th day
10	2	0.5	375	4	≤1/1000	-	200	2	-	-	-	-	-	-	-
11	3	0.5	375	4	<1/1000	>5<10	200	3	-	-	-	-	-	-	-
12	3	0.25	190	4	≤1/1000	≥1<5	200	-	3	-	1	<1/1000	<1/1000	1	Died from botulism on 4th day
13	3	10	7,500	4	≤1/100	>20<30	400-800	-	3	-	1	<1/1000	<1/1000	1	Died from botulism on 4th day
Totals	49							29	11	3	6			6	

five to six 24-hour periods. On the 8th to 11th day after immunization, they received injections suboccipitally of the botulinic toxin. Prior to the injection of the toxin, again the titer for the anti-toxin in the blood and liquiform was determined. In the blood, from 5 to 50 AE were found; in the liquiform, 1/1000 AE or ⟨1/1000 AE in one milliliter. After an active immunization, the quantity of anti-toxin in the liquiform reservoir of rabbits did not increase either.

These experiments also show the defensive ability of the central nervous system in rabbits from large doses of botulinic toxin, when the former are immunized with a botulinic anatoxin.

Control for all described experiments took the form of a constant checking of the Dlm botulinic toxin for rabbits during the suboccipital injection of the same.

It was established through the work of physiologists that the reservoir of liquiform possesses a movement in a caudal direction. This was corroborated by the research of P. N. Ulyanova (1930).

Consequently it was possible to assume that the toxin, introduced suboccipitally, was not able to manifest lengthy action on the central nervous system since it rapidly removed itself in a caudal direction into the blood where it was neutralized by the circulating anti-toxin.

In order to explain this problem, the following series of experiments were undertaken. We decided to check the results obtained by us by means of other methods for injecting toxin into

rabbits, through which the toxin would enter most fully into contact with the main part of the brain. Prerequisites of the test remained the same - blood was not to fall into the liquiform.

The first experiments were established with tetanus toxin.

We selected three methods for introducing the toxin into rabbits:

1) through a trepanated opening in the area of the main brain's hemisphere under the hard cerebral membrane on the day of operation (according to the Paster station method at the Mechnikov Institute in Moscow);

2) through the trepanated opening intra-cerebrally;

3) through the supra-orbital canal.

During the first two experiments, ten rabbits received intra-venous injections of 20 milliliters each (500 AE) of the anti-tetanus serum and after four hours 200 Dlm of the tetanus toxin subdurally through the trepanated opening. Seven rabbits remained healthy, one died from cerebral tetanus, and two from other causes.

Subsequent tests were made on nine rabbits which received intra-venous injections of 5, 2 and 1 milliliter of serum (125, 200, 100 AE) and after four hours the same method was used to introduce 200 Dlm of the toxin. Of the nine rabbits, only one died from cerebral tetanus; it had received five milliliters of the serum. These experiments show that ... some way 5, 2 and 1 milliliter (125, 200, 100 AE) of the serum protects the nervous system of rabbits from 200 Dlm of toxin during the sub-dural method of injection on the day of the operation. However this contradicts the data obtained by us in

the above described experiments, (Matveev and Sokolov, 1947). The serum doses of 5 and 10 milliliters (2,025 to 4,050 AE) in connection with a suboccipital injection of 200 Dlm toxin could not protect the central nervous system of the rabbits.

Subsequently, it was concluded that the results of these tests appear to be unconfirmable. Also the method of introducing the toxin into the rabbits under the hard cerebral membrane immediately following the trepanation must be considered unsuitable, since blood leaves the wound and neutralizes the toxin.

The second series of experiments was undertaken on four rabbits, into which the toxin was introduced intracerebrally through the trepanated opening according to the method of Van-den-Hoven and Decombe. First of all the rabbits were given intravenously two and one milliliters (200 - 100 AE) each of anti-tetanus serum, doses that could not protect the central nervous system in connection with a sub-occipital injection of 200 Dlm toxin and during four hours intracerebrally 200 Dlm tetanus toxin.

All rabbits in this series remained alive and healthy.

The third series of tests was also set up on four rabbits, into which toxin was also injected sub-durally through the canalis supro-orbitalis. These animals were given intravenously two and one milliliters each (200-100 AE) of serum and during four hours 200 Dlm of toxin.

All rabbits, similarly to those in the second series of tests, remained healthy. In these two series of experiments, the animals stayed healthy because during injection of the toxin into the tissue

of the brain or into the canalis suproorbitalis there took place a wounding of the brain vessels. Blood emanated from the latter and, since blood contained anti-toxin, the toxin underwent neutralization. In support of this conclusion is the fact that the quantity of serum which the animals received in these experiments does not protect the central nervous system from 200 Dlm of tetanus toxin when it is injected suboccipitally (Matveev and Sokolova 1947).

Consequently, from these three series of tests, we can come to the conclusion that the sub-durally toxin injection on the day of trepanation, intracerebrally and into the canalis suproorbitalis does not appear to be of any value for testing the reactiveness of the nervous system in xx immunized animals.

In order to obtain unchallengable data showing whether the toxin proceeds in a caudal direction, when it is injected into the cisterna magna and what the role of this phenomenon is for protection of the central nervous system in immune animals, we utilized a method for introducing tetanus and botulinic toxins through a trepanated opening in the area of the brain hemisphere under the hard brain membrane. The toxin was injected through the undamaged hard membrane of the brain on the day following the operation, when the hemorrhage from the diploetic veins had ceased. In such conditions, the toxin was located in contact with the brain tissue for a longer period. Leading it in a caudal direction could play no substantive role. The absence of protection to the main part of the brain by the anti-toxin circulating in the blood should have brought about the death of the animals from tetanus and botulism.

Fresh rabbits were taken and submitted to trepanation operations, after which the blood and liquiform were observed for AE content. On the following day serum was introduced intravenously, and during four hours toxin was injected into the animals by piercing the hard brain membrane.

The first series of experiments was conducted with tetanus toxin. Five rabbits received 2.5 and 5 milliliters of anti-tetanus serum (1250 - 2025 AE) and during four hours 200 Dlm of the toxin. All rabbits died from tetanus.

Subsequent tests were made on 10 rabbits which received ten and two milliliters (8,100 AE) of the serum and during four hours 200 Dlm of the tetanus toxin. Of the ten rabbits, two remained healthy, six died from cerebral tetanus, and two died from other causes.

In other experiments conducted on three rabbits, it was established that even the injection of 20 milliliters (16,000 AE) of serum did not protect the central nervous system of the animals from 200 Dlm of toxin which had been introduced subdurally in the area of the hemisphere. The obtained results were significant, also because in our earlier experiments (Matveev and Sokolov, 1947) after injecting 20 milliliters (8,000 AE) and 40 milliliters (10,000 AE) of the serum during a sub-occipital introduction of 200 Dlm of the tetanus toxin, all rabbits remained alive.

During subsequent tests, we reduced twice the dose of tetanus toxin.

On the day following the trepanation, the animals received intravenous injedtions of 20 milliliters each (16,000 AE) and during

four hours 100 Dlm of toxin was introduced under the hard brain membrane. All six rabbits stayed healthy. The dose of serum of 10 milliliters (8,000 AE) could not protect all animals from 100 Dlm of the toxin, introduced by this method (of the six rabbits, one died from tetanus). A dose of 20 milliliters (4,000 AE) protected from this amount of toxin only half of the animals (in the tests, we used different series of serum containing various amounts of AE in one milliliter).

These experiments show that during suboccipital injection of the tetanus toxin, the latter's movement in a caudal direction may play a part in the protection of the central nervous system of immunized animals.

In the event that the toxin is injected under the hard brain membrane in the area of the brain hemisphere, there takes place a lengthier contact of the toxin with the nervous system. In these tests, it was protected by the serum from smaller doses of the toxin. If during the sub-occipital injection of the toxin, 10 - 20 milliliters (8,000 - 16,000 AE) of serum protected the nervous system of rabbits from 200 Dlm, then when introducing the toxin under the hard brain membrane in the area of the brain hemisphere, these doses protected animals only from 100 Dlm of the tetanus toxin.

The obtained results were checked in experiments with the botulinic toxin. In the course of five tests, the rabbits underwent a trepanation in the area of the hemisphere; on the following day, they received intravenous injections of 3, 1.5, 1.0, 0.5 milliliters each of anti-botulinic serum (2,200; 1,100; 750; 375 AE); during four hours, injections were given of 200 Dlm toxin

under the hard brain membrane in the area of the brain hemisphere. The anti-toxin titer in the blood of animals was more than 10 and less than 30 AE, and in the liquiform cystern it was 1/1000 AE.

Of the 15 rabbits, 14 remained alive and one died from an unknown cause. As a control group for these tests, we had seven rabbits which received the same amount of serum; the toxin was injected sub-occipitally. All of these animals remained healthy (see Table 20).

The experiments on the rabbits, immunized with botulinic ana-toxin, corroborated our data that have been described above.

Of the seven animals that received sub-durally in the area of the brain hemisphere from 200 to 2,000 Dlm of botulinic toxin, two died from botulism (500 and 2,000 Dlm), one from an unknown cause, and four remained healthy.

In all of the rabbits, the quantity of anti-toxin in the liquiform cystern amounted to less than 1/1000 AE; in the blood, it was from 5 to 50 AE. In the rabbit that died from 500 Dlm, the anti-toxin titer in the blood was considerably lower than in the healthy animals that received 200 Dlm of the toxin.

Observations conducted with the botulinic toxin did not give any results that would corroborate the data obtained with the tetanus toxin: about its fragmentary movement in a caudal direction during injection into the cisterna magna and the significance of this factor for the reactiveness of the central nervous system toward botulinic toxin in immunized animals.

The experiments undertaken upon passively and actively immunized animals show that the method of introducing the botulinic toxin

Table 20. Changes in the Reactiveness of the Central Nervous System of Rabbits during Passive Immunization against Botulism. Sub-dural Introduction of the Toxin on the Second Day after Trepanation.

Experiment											Control							
Number of experiment	Number of rabbits	Intravenous injection of serum (in milliliters)	Quantity of AE	Hours toxin injection lasted	Quantity of AE prior to introduction of toxin		Toxin (in Dlm) injected sub durally on second day after trepanation	Number of rabbits after toxin shot			Number of rabbits in control	Quantity of serum injected intravenously (in milliliter)	Quantity of AE prior to introduction of toxin		Sub-occipital introduction of toxin (in Dlm)	Number of rabbits after toxin shot		
					in liquiform	in blood		Remained healthy	Died from botulism	Died from other			in liquiform	in blood		Remained healthy	Died from botulism	Died from other
1	4	3.0	2,200	4	<1/1000	=30	200	4	-	-	1	2,200	<1/1000	=30	200	1	-	-
2	4	1.5	1,100	4	<1/1000	>15<20	200	3*	-	1	-	-	-	-	-	-	-	-
3	2	1.5	1,100	4	<1/1000	>15<20	200	2	-	-	2	1,100	<1/1000	>15<20	200	2	-	-
4	3	1.0	750	4	<1/1000	=15	200	3	-	-	2	750	<1/1000	=15	200	2	-	-
5	2	0.5	375	4	<1/1000	>10<15	200	2	-	-	2	375	<1/1000	>10<15	200	2	-	-
Total	15							14	-	1	7					6	-	1

* One of the rabbits became ill with botulism but remained alive.

plays no substantive part. One and the same very small quantities of serum (o.5 milliliters, 375 AE), injected intravenously, protect the central nervous system of rabbits from very large doses of the toxin (200 Dlm) injected sub-occipitally and sub-durally in the area of the brain hemisphere.

After such a manner, the obtained experimental data show that the condition of the central nervous system during passive and active immunization against botulism does not differ from that condition in which it remains during immunization against tetanus. Disregarding the absence of anti-toxin in the liquiform cystern of animals immunized against botulism, their central nervous system stays in an areactive condition toward the action of very large botulinic toxin doses introduced by the sub-occipital or sub-dural methods. This shows that the anti-toxin, found in the blood, penetrates through the capillary walls into the intra-mural liquiform that directly washes the brain cells. For this reason, the protective qualities of the central nervous system during immunization against botulism and tetanus can be explained as follows: the toxin located in direct contact with the nervous system is neutralized by anti-bodies, situated in the nerve cells in the form of sessile receptors as well as anti-bodies found in the intramural liquiform.

However, if we introduce sub-occipitally or sub-durally a very large quantity of tetanus or botulinic toxin, the animals will die, because the anti-toxin situated in the intramural liquiform appears to be insufficient for neutralization of the injected toxin.

The results of research on botulinic toxin show that during the introduction of larger quantities sub-occipitally or sub-durally, very small quantities of serum are sufficient for protection of the central nervous system.

The anti-tetanus serum, which was injected into animals in our tests, contained a quantity of AE neutralizing 1,000 Dlm of the tetanus toxin for mice. During the sub-occipital or subdural injection of 100 - 200 Dlm into a rabbit, this quantity of toxin comprised 20,000 - 40,000 Dlm for mice. Only large quantities (10 ⊖ 20 milliliters; 8,000 - 16,000 AE) of very strong anti-tetanus serum protected the central nervous system from larger doses of tetanus toxin. We assume that this is explained by the sensitivity of the nervous system to tetanus toxin and that for its protection a considerable concentration of anti-bodies is required in the intramural liquiform.

A completely different phenomenon was observed in the tests on the botulinic toxin. Very small quantities of anti-botulinic serum (0.5 milliliters; 375 AE), i.e. ten times smaller than the anti-tetanus serum, introduced intravenously protected the central nervous system from very large doses of toxin equal to 200 - 500 Dlm for the rabbit or 100,000 - 250,000 Dlm for the mouse. Apparently, this less successful serum therapy of tetanus in comparison with botulism is explained by the greater sensitivity of the nervous system toward the tetanus toxin. However this can be stated only in terms of a hypothesis.

In further experiments it was found how to explain the protection of the central nervous system during immunization against

botulism and tetanus from large toxin doses (up to 200 Dlm) at the same time that the cystern of liquiform contained no anti-toxin.

In the course of the preceding experiments we had laid down the hypothesis that the anti-toxin found in the blood penetrates through the capillary walls into the intramural liquiform which directly washes the brain cells. For this reason, the protection of the central nervous system during immunization against tetanus and botulism may be explained as follows: the toxin, situated in direct contact with the nervous system, is neutralized by anti-bodies which are found in the nerve cells in the form of sessile receptors as well as anti-bodies located in the intramural liquiform.

Freund (1930) established that the agglutinins may be extracted from the main part of the brain and the spinal cord of rabbits, actively and passively immunized against typhoid fever. The penetration of anti-bodies into the brain took place very rapidly during 15 minutes, and in the liquiform they were found during several hours after injecting the immune serum into the blood. He finds it doubtful that the anti-bodies penetrate into the brain through the spinal-cord fluid, because the titer of agglutinins is larger in the brain than in the liquiform and because they penetrate more rapidly into the brain.

Stolchenova (1945) studied the distribution of the immune serum in the organism of experimental animals and obtained contradictory results. She injected typhoid-fever serum intravenously into rabbits, washed the organs with a physiological solution through the vessels, and by means of filtrates set up an agglutination

reaction in the organs. In the main part of the brain and in the spinal cord, the agglutinins were not discovered in a single case.

In the literature on the field, we could not find any mention about the discovery of anti-toxins in the tissue of the central nervous system. In order to explain the possibility of penetration by the tetanus and botulinic toxins into the tissue of the main part of the brain, we set up the following experiments (Bulatova and Matveev, 1949).

The rabbits received intravenous injections of anti-botulinic and anti-tetanus serum. During four hours, at first taking the liquiform by means of sub-occipital piercing, the animal was given a narcosis; tubes were inserted into the a. carotis from both sides, the head was cut off, and the Ringer-Lokk solution was allowed to pass through for a period of 1.5 hours (during that time, between three and four liters of the solution went through the vessels). The fluid, leaking from the veins during 1.5 hours from the time the solution started passing through, was collected and titrated for the presence of anti-toxins. Such a lengthy passing of the fluid through the vessels is necessary in order to wash all anti-bodies out of them. Of course, in part also anti-bodies that had penetrated from the blood intc the central nervous system were washed away.

After completion of the test, the brain was absolutely without blood everywhere. A suspension with a double volume of the physiological solution was made from it; at night, this was placed into a refrigerator. On the following day, it was filtered through a talcous filter and titrated for the presence of anti-toxins.

We established seven tests with the anti-tetanus serum. The rabbits received intravenously 4,000 AE each. Titration of the liquiform cystern, of the Ringer-Lokk solution, passed through the brain vessels, and the filtrates of the brain suspension in 1/1000, 1/100 and 1/10 AE in one milliliter took place. In these seven experiments, only one case of the anti-toxin titer appeared to be the same in the Ringer-Lokk solution as in the brain suspension filtrate.

In the course of six tests the anti-toxin titer in the brain suspension filtrate was ten times larger than the anti-toxin titer in the Ringer-Lokk solution that had been passed through the vessels of the brain. In the liquiform cystern, the anti-toxin titer always remained $<$ 1/1000 AE in one milliliter.

Eight experiments were set up with the anti-botulinic serum.

During the first three tests on rabbits, they each received intravenously 7500 to 3750 AE. Identical titers were discovered in the filtrate from the brain suspension and in the Ringer-Lokk solution. During subsequent experiments, the rabbits were given smaller quantities of serum but such that would protect the rabbits from 200 Dlm of the botulinic toxin during sub-occipital or sub-dural injection of the same.

In the course of five tests, the rabbits received intravenous injections each of 0.5 - 1 milliliter of serum (375 and 750 AE). The results of titration showed that the anti-toxin titer in the Ringer-Lokk solution was always 5 to 10 times smaller than that in the filtrate from the brain suspension. In the liquiform cystern, the anti-toxin titer always remained $<$ 1/1000 AE in one milliliter.

These observations, conducted with anti-tetanus and anti-botulinic serum, show that regardless of the absence of anti-toxin in the central liquiform it maintains itself in the nerve tissue after it has been washed out completely from the brain vessels with a Ringer-Lokk solution (Table 21).

Table 21. Penetration of Anti-toxin into the tissue of the Brain in Rabbits during Intravenous Injection of Anti-Tetanus and Anti-Botulinic Serum

Number of rabbit	Quantity of serum injected intravenously into rabbit (in milliliters)	Quantity of AE	Quantity of AE found in the cystern of the liquiform	Quantity of AE found in the Ringer-Lokk solution, passed through the brain vessels	Quantity of AE found in the brain suspension after washing the vessels with Ringer-Lokk solution
			Anti-Tetanus Serum		
1	20	4,000	<1/1000	=1/1000	>1/100<1/10
2	20	4,000	<1/1000	>1/1000<1/100	>1/100<1/10
3	20	4,000	<1/1000	=1/100	>1/100<1/10
4	20	4,000	<1/1000	>1/1000<1/100	>1/100<1/10
5	20	4,000	<1/1000	>1/100 <1/50	>1/100<1/50
6	20	4,000	<1/1000	=1/100	>1/100
7	20	4,000	<1/1000	<1/1000	=1/100
			Anti-Botulinic Serum		
1	10	7,500	<1/1000	>1/100 <1/10	>1/100<1/10
2	10	7,500	<1/1000	>1/50 <1/10	>1/50 <1/10
3	5	3,750	<1/1000	>1/100 <1/50	>1/100<1/50
4	1.0	750	<1/1000	=1/1000	>1/000<1/10
5	1.0	750	<1/1000	<1/100	=1/100
6	0.5	375	<1/1000	=1/100	>1/100<1/10
7	0.5	375	<1/1000	=1/1000	>1/100<1/10
8	0.5	375	<1/1000	=1/1000	=1/10

Our previous experiments, in which we established the areactiveness of the central nerve system toward tetanus and botulinic toxins in actively and passiyely immunized animals, appear to be circumstantial evidence for the penetration of anti-bodies through the capillary walls into the central nervous system. Tests set up with the washing of brain vessels indicated the presence of anti-toxin in the filtrate of the brain suspension, which appears to be direct proof of the penetration by anti-toxins from the blood-carrying vessels into the tissue of the central nervous system.

In such a way the mechanism of areactiveness toward toxin by the central nervous system in animals, during immunization against tetanus and botulism, depends upon the penetration of anti-bodies toward the brain cells in the intramural liquiform. The anti-bodies penetrate through the capillary walls. It is possible that they are located on the nerve cells in the form of sessile receptors, defending the former from the toxin injected into animals by the sub-occipital or sub-dural methods. During an active immunization, the possibility is not excluded for an immunized reconstruction of the nerve cells themselves. However this hypothesis requires itself to be proven.

From the above described experimental observations, clear and practical conclusions flow.

During the serum therapy for botulism and tetanus, it is necessary to inject as early as possible intravenously large quantities of serum. In order to avoid shock, the intravenous injection should be commenced with small quantities of serum (5 - 10 milliliters), and then during two to three hours with 50 - 100 milliliters.

The patients should also receive injections of the serum intramuscularly several times of 50 - 100 milliliters each.

Such a method of serum therapy for tetanus, according to the data of Sokolov (1943) and Ishkhanov (1945), was justified very well in practical application.

During the intravenous introduction of large quantities of serum, good medical results are obtained in connection with the rapid penetration of the anti-toxin through the capillary walls in the intramural liquiform. In the event that the toxin enters the liquiform, the former undergoes neutralization.

A late injection of the serum provides poorer therapeutic results, because the toxin is able to make a strong connection with the central nervous system and the anti-toxin can not split the toxin away from the nerve tissue and consequently neutralize.

3. Anti-Bacterial Immunity

The study of the basic phases in the development of anti-toxic immunity toward botulism has yet to introduce full clarity into a presentation of its existence, especially concerning its natural immunity.

As has been shown in the foregoing, with the aid of established facts it was impossible to explain the stability of certain persons and animals toward the botulinic toxin. During botulism epidemics in various countries, many phenomena observed were not understood. At the same time that certain members of a family, having eaten products infected with the toxin, became ill with a serious form of botulism which often had fatal consequences - others stayed completely well or were sick with a light form of

botulism. A considerable number of such cases have been described in foreign as well as in our own native literature. During the study of fish poisoning at Astrakhan, Sokolov (1886-1893) observed a large number of persons who remained healthy after eating fish that had been the cause of a series of poisonings. According to the data of Sokolov, children appeared to be less resistant to the botulinic toxin. Among 13 poisoned children in ages up to 16 years, eleven (85 percent) died; of the 137 adults who were poisoned, only 46 (33 percent) died. Wilbur and Ophules (1914) described a botulism epidemic at Standford, where 24 students ate some bean salad; 12 of them became ill, the others stayed well. As Geiger, Dixon and Meier announce in their monograph of the epidemology of botulism (1922), a mother and son both ate canned asparagus; the mother died from botulism, but the son remained healthy. Among eight persons who partook of canned beans, seven became ill but one was not affected. Fridman, Lorber and Silberman (1936) described several similar cases, where not all of those who ate poisoned fish in their food became sick. A mother and daughter ate smoked herring; the mother died from botulism, whereas the daughter did not become ill. Thirteen persons ate some fish; three of these came down with sickness, the remaining stayed well. Roe containing botulinic toxin was eaten by seven individuals, but only two became ill. One family of eight persons partook of salted sturgeon in their food, in the course of which all of them ate in the same quantity; only four came down with botulism. Fish from the same lot was eaten by a husband and wife; the husband died from botulism, the wife was well.

Especially interesting material was released by Minervin and Kotlyarevskaya (1937). According to their data, in 1933 a total of 172 persons in 61 families ate fish roe containing botulinic toxin and microbes. Of these 114 persons came down with botulism in various degrees, many of these cases ending in fatalities. The remaining members of the family were well. This can possibly be explained by the cluster-like distribution of the toxin in the product at a given instance.

Certain researchers attempted to explain the insensitivity toward toxin by individual persons through the presence in their blood of an anti-toxin for the poison of the botulism bacillus. The experiments of Meier and Geiger (1921), Kolimen (1922) provided negative results. Even in the blood of those who recovered from botulism, they could not find any anti-toxin.

Minervin and Kotlyarevskaya (1937) occupied themselved with the study of this problem. They write: "It is possible to assume that anti-bacterial immunity plays a part in the pathogenesis of botulism in man. However the materials available in this connection to date are still insufficient for a solution of this problem and require further collection and study."

These authors make the hypothesis that the resistance of man to botulism may be related to anti-bacterial immunity. They have based their conclusions upon experimental observations in which guinea pigs, immunized with botulism microbes, appeared to be considerably more resistant to the disease of botulism.

The question arises: by what means can anti-bacterial immunity toward botulism develop in the human being? Apart from this, it is

necessary to explain whether there is at all available data supporting the possibility for the development of such an immunity.

Facts were presented in the foregoing which testified to the very wide distribution in nature of the botulism bacillus, especially in certain of its types. Research conducted in different parts of the USSR shows that type A is more widely distributed in nature than is type B.

In connection with the fact that the botulism microbe is often discovered in the soil, on fruit and vegetables there is a large possibility for the infection of food products with this bacillus. This enables it to penetrate into the intestines of the human being together with contaminated food. A study of the intestinal content in healthy persons for the presence of the botulism bacillus supports the possibility of a similar path for the penetration. Tanner and Dek (1922) obtained two cultures of type B from 10 specimens of feces of healthy people. Graham and Warger (1921) discovered the microbe of botulism in the feces of a laboratory aide who had never been sick with botulism. A similar case is reported by Fridman and Lorber (1937).

The possibility for the spores of the botulism microbe to penetrate into the human intestines as well as those of animals and from there into the internal organs is no longer open to doubt at the present time. The botulism stimulant was discovered in the internal organs (Shapiro and Nikolenko, 1937) during the dissection of cadavers immediately upon death. There is also data in the literature on the field about the discovery of the botulism microbe in the intestines of botulism patients (Fridman and Lorber, 1937).

However of special interest is the research on the collection of agglutinins and their concentration in the direction of the botulism bacillus in the blood of recovered patients. Geiger, Armstrong, Story, Scott (1919) discovered the agglutinins in the blood of recovered persons on the 14, 18 and 26th day in a dilution of 1:40 and 1:320. Nikolenko and Burnos (1937) presented considerable material on this problem. They studied that presence of agglutinins for the botulism microbe in 80 serums on people ill with botulism or suspected of this disease. Blood samples were taken at various times after the disease - from three days to three months. In many patients and those recovered from botulism, the agglutination reaction showed itself to be positive when the serum was diluted 1:200 and in certain cases 1:400 and 1:800. In one instance, they observed a positive agglutination reaction with the botulism bacillus in a healthy man at a dilution of 1:400. All of these data indicate the ability of the botulism microbe to penetrate from the intestines into the internal organs of man and facilitate the production of agglutinates.

Miss Fridman (1941) observed 532 serums of healthy persons for the presence of agglutinins toward the botulism microbe. It appeared that 124 of the serums from healthy individuals contained agglutinins toward this microbe in various titers. In certain of the individuals the agglutinins were discovered when the serum was diluted 1:1,600 to 1:3,200. A positive agglutination was obtained with the botulism microbe type A but very rarely with type B. As is known, in the development of anti-bacterial immunity a part is played not only by bacteriolysins, opsonins, tropins, precipitins

but also by agglutinins. For this reason we set up experiments for the explanation of the presence of agglutinins for the botulism bacillus in healthy people, domestic animals and experimental animals.

In order to establish these tests, we utilized the serums taken from donors for the purpose of checking the reaction in them of the Wasserman test. The agglutination reaction was conducted at first with the botulism microbe type A and B, subsequently only with type A.

In connection with the fact that 200 human serums gave no agglutination reaction to badillus botulinus type B in all cases, it was not used any more with this anti-gene.

The anti-gene for the reaction was prepared by means of making a culture with the botulism microbe in boullion without sausage meat, located in large bottles having from five to eight liters in volume. The culture was made copious, after which it was maintained in a thermostat for 10 to 12 days. Usually during that time the bacteria, after a violent growth, subsided to the bottom of the bottle. The top layer of the broth was poured off, whereas the sediment consisting almost completely of bacterial cells and spores was drawn off into small containers. Then the bacterial mass was diluted in a 0.5 percent solution of chloride of sodium up to 20 milliard badterial bodies in one milliliter according to the standard B. coli, and one percent of formalin was added to it. The antigene was kept in a thermostat with formalin three to four days, after which it was protected in a refrigerator all of the time. Prepared in such a way, the antigene could be utilized for the setting up of a reaction during the course of a long period. Prior to the experiment,

the human serum was diluted not with a physiological solution as is usual but with a 0.5 percent solution of sodium chloride in order to avoid spontaneous agglutination. To each test tube was added from one to two drops of antigene, after which the test tubes were placed in the thermostat for two hours. Then the results were noted. After this the test tubes remained on the table, and the results were checked a second time during 18 to 20 hours. In the majority of cases, they did not change. Periodically the specificity of the anti-gene was checked by means of setting up an agglutination reaction with the typical specific serums from the botulism microbes type A and B.

It was part of our work to check 500 serums from healthy individuals for the presence of agglutinins for the botulism microbe. As is apparent from Table 22, a total of 212 serums gave a positive agglutination reaction with the anti-gene of type A. In 179 cases the agglutnins were maintained in small quantity. The reaction was positive with a small dilution of the serum. The remaining 39 personal serums contained agglutinins in considerable quantity. A positive reaction in a dilution of 1:400 was given by 14 serums; of 1:500 by five; of 1:800 by 13; of 1:1,000 by one; and of 1:1,600 by three serums.

As has been shown above, such data was also obtained by Nikolenko and Burnos as well as by Fridman when they studied the reaction of agglutination with the botulism bacillus in people. All of this has considerable significance for an understanding of natural immunization of a human being to botulism. Apart from the presence in the blood and agglutinins, which appears to be in part an indication of development on the part of an anti-bacterial immunity in persons

having a high titer of agglutinins for the botulism microbe, the blood contains also other anti-bacterial antibodies of this bacillus. It is possible that these people have an areactiveness to the botulinic toxin by the cells in the organ tissue.

Table 22. Reaction of Agglutination during Symptomless Botulinic Infection of Human Beings and Animals

Serum	Number of serums being checked	Negative agglutination reaction	Positive reaction to agglutination												
			1:50	1:100	1:200	1:400	1:500	1:800	1:1,000	1:1,200	1:1,400	1:1,600	1:1,800	1:2,000	Totals
Agglutination Reactions with Human Serums and Stimulant of Botulism															
Of man (from donors)	500	288	86	57	36	14	13	5	1	0	-	3	-	-	212
Agglutination Reactions with Serums from Domestic Animals and Botulism Stimulant															
From pigs	50	10	2	9	3	6	-	13	4	2	0	0	1	0	40
Big cattle	100	29	3	12	15	18	-	15	4	1	2	0	1	0	71
Agglutination Reactions with Animal Serums during Experimental Symptomless Botulism															
Rabbits	62	29	1	5	6	3	3	5	-	-	5	-	5	-	33
Guinea pigs	31	7	2	3	5	-	11	-	1	-	2	-	1	-	24

The serums of human beings, providing a positive agglutination in dilutions of 1:800 and higher, were used for setting up a neutralization reaction with the botulinic toxin. In all cases, it was found to be negative; 0.5 milliliters of the serum would not even neutralize one lethal dose of the botulinic toxin for mice. Only one serum, giving a positive agglutination in a dilution of 1:1,600, precipitated a postponement of the mouses death for three to four days. These experiments clearly showed the absence of anti-toxin in the serum of people during a high titer of the agglutinins.

The presence of agglutinins in the serum of healthy individuals may be explained only by the fact that, in connectionx with a considerable distribution of the botulism microbe in nature, there takes place often contamination of various products with the spores of this microbe. The data, enumerated above after observation of the soil, support this hypothesis. In the event of eating with food such products, at times the human intestines are entered by considerable quantities of spores which penetrate into the organs and cause the formation of agglutinins. If the spores of the botulism bacillus only rarely fall into the food of man, the agglutinins are absent from the serum or are available only in small quantities. The content of agglutinins of the botulism microbe may exist in large quantities in the serum of certain healthy individuals, and this is apparently related to the fact that at times the persons ate products heavily infected by the spores of this microbe.

On the basis of the data from literature in the field and from experiments, the possibility of the botulism microbe going

from the intestines into the organs may be considered as fully established. It is clear that the botulism microbes, situated in the organs, cause a determined reaction by the tissue cells on their presence; as already has been shown, this may lead not only to the formation of agglutnins but also to the areactiveness of the organ cells toward botulinic toxin.

In support of the enunciated position, we also have the results from studying the reaction of agglutination with the botulism microbe in domestic animals.

We selected 50 serums from pigs and 100 serums from large cattle (cows) for our experiments; the animals were characterized by a small sensitivity toward botulism. It is known that pigs are carriers of tremendous doses of the botulinic toxin, without showing any symptoms of the disease. During botulism epidemics among horses in collective farms, which were caused by fodder from silos, the cattle and pigs did not become sick even after feeding from the same silos for considerable lengths of time.

During the time of the German occupation of France from 1940 to 1944, there were 500 epidemics of botulism involving over 1,000 human beings. The cause for the botulism disease in 93 percent of the cases was pork. Studying this problem, Legr, Dzheramek and Levaditi (1944, 1945) came to the conclusion that pigs during their lives may be infected with the spores from the botulism bacillus, especially in those cases when they are fed on throw-aways. Legr and Dzheramek discovered large quantities of spores from the botulism microbe in the meat of one pig immediately after it had been slaughtered. In connection with such facts, the study of

anti-toxins and agglutinins in these animals was very important for an explanation of the mechanism in the development of a natural anti-bacterial immunity.

The experiments were set up with the application of the previous methodology; the antigene used in the agglutination reaction with human serum was also applied here. The results of the observation appeared to be completely different. The agglutination with the serum from pigs was positive, with the botulism microbe of type A in 80 percent, and also in 20 cases (40 percent) with a dilution of the serum from 1:800 to 1:1,800 (Table 22). Of the 50 serums, a negative reaction was given by only 10. The serums from cattle provided identical results. Among 100 serums, a positive reaction was obtained in 71 (71 percent). When diluting the serums from 1:800 to 1:1,800 the reaction was positive in 23 instances (23 percent).

The anti-toxin was not discovered in the serum of these animals.

The presence in large quantity of agglutinins in the blood of animals is explained by the type of food they eat. When feeding on various fodder, large quantities of spores from the botulism stimulant enter the animal organism. The presence of the botulism bacillus in the intestinal contents of pigs and cattle was established by the research of Easton and Meier (1924). In the event of penetration by the spores of this microbe from the intestines into the organs, there takes place an immunization - in animals, not only agglutinins are produced but also a resistance to the toxin from the botulism bacillus.

The high degree of stability toward the botulinic toxin shown by the mentioned types of animals has been established long ago. It was possible for us to discover in their blood large quantities of agglutinins for the stimulant of this disease, which also indicates the development of an immunity.

The possibility of the development of an anti-bacterial immunity toward botulism on the part of animals in natural conditions of existence is also supported by experimental data.

As has been shown by Meier, Leikhs at first obtained an agglutination with the serum of animals immunized by botulinic toxin that contained bacteria. Bronfenbrenner, Shlezinger and Kalazans (1921) showed that the serum of animals immunized from the botulism microbe agglutinate this microbe well. Shengolts and Meier (1923) immunized 69 rabbits with a dead culture of the botulism microbe; of these, 46 animals provided a good agglutination serum in separate instances with the titer up to 1:4000 and 1:10 000. With the serum of eight rabbits, the agglutination was weak; and the serums of 15 rabbits did not contain any agglutinins whatsoever. Similar results were obtained by Sterin and Dek (1923). These authors note the formation of agglutinins in very large quantity during the immunization of rabbits by the spores of the botulism bacillus; the titers of serums in such conditions were considerably increased, but a part of the animals died from botulism notwithstanding. Good serums, agglutinating the botulism microbe, were obtained in the USSR by Zelevinskaya (1935), Matyash (1940), Matyash and Askalonov (1941).

In order to study the role of anti-bacterial immunity in relation to the protection of the organism from the disease of

botulism, very interesting observations were made by Minervin and Kotlyarevskaya (1937). It appeared that guinea pigs immunized with the botulism microbe, during the absence from their blood of anti-toxin, were possessed of a considerable stability in regard to the infection of botulism which was caused in them by a sensibilization of the toxin. These authors consider it possible that an anti-bacterial immunity toward botulism may exist in human beings as well as in animals and that this immunity may have been introduced through a natural path in the process of feeding on infected products.

During the time devoted to the experiments described in the foregoing, we also studied agglutinins in the serums of animals contaminated by the spores of botulism microbes. The concentration of these anti-bodies was observed in rabbits and guinea pigs when they were infected subcutaneously with spores and also through the mouth. The appearance of agglutinins in the blood of animals, infected with the spores of the botulism bacillus through the mouth, testify to the fact that they penetrate through the walls of the intestines into the organs and cause the development of an anti-bacterial immunity.

The agglutination reaction, during the infection with spores, was positive for 33 rabbits among 63 and for 24 guinea pigs out of 31 (Table 22). The agglutinins were discovered in various titers; in individual cases the serum provided a positive reaction in dilutions of 1:800 and 1:1000. In view of this fact that the animals in their majority were infected once with a small quantity of spores, the high titer of the agglutinins may be explained only by the development in them, as we assume, of a symptomless botulinic infection.

In such a manner, the data contained in literature on the field and the results of our own experiments leads us to the conclusion thatbthere is a possibility for development of an anti-bacterial immunity to botulism on the part of human beings as well as animals.

Under the heading of causes for contamination of cartilaginous fish by the botulism stimulant, it appears that they may be met with in individual lots of the product which has been considerably contaminated by the spores even in the absence of the toxin. When eating such products in the form of food (usually after cooking, which does not kill the spores) people apparently develop a symptomless infection that usually ends fortunately without any visible clinical symptoms. During the developmental process of the infection, agglutinins are produced, and there takes place a transformation in the reactiveness of the tissue cells toward the botulinic toxin. The development of cellular immunity toward botulism a pears to be the cause of stability on the part of injdividual persons to the botulinic toxin.

Apparently the anti-bacterial immunity and the areactiveness on the part of tissue cells protect in individual cases the human being and animals from the botulism disease. These means for defense appear to be the basic ones during the penetration of spores of the botulism bacillus into the organs at the time contaminated food or fodder is being eaten.

C O N C L U S I O N

Summarizing the above described data found in the literature on the field and in experiments, we are able to make a series of conclusions on their basis.

The spores of the botulism stimulant possess an exclusively greater stability toward the action of chemical and physical factors. Under the influence of a high temperature, they may transform themselves inside of cultures into "dormant spores" and not develop for a long period of time.

Food products (meat, fish, fruit, vegetables in cans, etc.), contaminated with the spores, even after freezing may become toxic in connection with the destruction of certain parts of the spores under the influence of low temperatures. Spores introduced into the organism of the human being and animals spread out, grow into vegetative forms with the formation of toxin, and cause changes in the reaction by cells as well as the production of agglutinins and antitoxins.

The botulism stimulant is capable of producing a very strong toxin in the organism of man and animal through food products in an artificial medium.

The problem of transforming toxigenous strains into nontoxigenous ones under the influence of high temperatures has been studied inadequately.

The concept that botulism appears to be a very rare disease does not correspond with reality. This is contradicted by the wide distribution of the botulism stimulant in nature and also by the

quite frequent discovery of it in food products. Apart from this, not all cases of botulism are diagnosed.

Warm-blooded animals play a considerable bole in the distribution of the botulism microbe on the earth's surface and in the contamination of the soil. Wild birds, making flights over long distances, rodents, and also domestic animals add to the spreading of the stimulant in nature. The statement that the botulism bacillus appears to be the natural inhabitant of only the soil, and even exclusively virgin soil, is without any kind of serious scientific foundation.

The problem of distribution on the territory of the USSR of individual types of the botulism stimulant requires further study: this is of considerable importance for the perfection of serum therapy against botulism.

In our country during the pre-revolutionary period and in the first years after the revolution, the principal factor causing the botulism disease in human beings was cartilaginous fish (various kinds of sturgeon). At the present time, a case of botulism in the USSR after eating cartilaginous fish salted industrially is very rare. Botulism is always still observed after the consumption of cartilaginous fish salted at home and smoked privately. Of great importance in the sharp decline of botulism in our coutry were the studies by Soviet microbiologists, who established the causes favoring the contamination of cartilaginous fish by the botulism microbe. Infection of the fish may take place not only by endogenous means - from the intestines, but also exogenously - from the outer medium, during poor sanitary conditions of processing, transporting and preserving the fish.

Bacteriological control over the cartilaginous fish, infected by the spores from the botulism microbe, showed that during its con-

sumption in food, even after cooking, the development in human beings of a symptomless botulinic infection is possible. The results of bacteriological control corroborate experimental observations on rodents (mice, grey rats, guinea pigs, rabbits). Grey rats ate fish infected with the spores of the botulism microbe, and the latter easily penetrated through the wall of the intestines, causing a contamination of the organ tissues in these animals. The same results were obtained also in other animals during their infection per mouth by the botulinic spores. Analogous phenomena were established in the cadavers of people who died from botulism; during the few hours after their death, the microbes of botulism were found many times in their organs.

Cartilaginous fish, contaminated with the spores from the botulism microbe, after thermal processing apparently is unable to cause clinically visible cases of botulism, despite the fact that the spores are not destroyed during such processing. However the absence of clinical symptoms of botulism does not provide the basis for the statement that small quantities of spores from the botulism microbe, falling into the human organism by consuming cartilaginous fish infected with spores, are completely harmless.

It can be said without any doubt that the microbe is conditioned to live in the organism of warm-blooded creatures that possess clearly expressed and stable toxigenous properties; when found in the organs of man or animal, it should cause specific changes in the organism.

The consumption in food of products, in which the presence of spores from the botulism bacillus have been established, may be safe only after sufficiently prolonged thermal processing that fully guarantees the destruction of the spores.

The results from the study of processes which take place in the organism of experimental animals (mice) during the presence of an infection, but in the absence of visible clinical symptoms of botulism, can be brought down into the following theses:

1. The botulism stimulant possesses the capability of penetrating from the alimentary tract into the organs of animals (liver, spleen), causing contamination of the organism. As a result there develops a symptomless botulinic infection which, after a surgical trauma, may be transformed into a clinical visible one ending in the death of the animals from botulism.

2. In the organs of animals, the botulism microbe may be located for long periods of time (up to 92 days) without losing its toxigenous properties and the capability of multiplying in the organism of warm-blooded creatures.

3. Toxin, gaining access to the alimentary canal of animals together with spores, similarly to the surgical trauma paralyzes the protective abilities of animals, contributes to the multiplication of the botulism stimulant and the production of new toxin, as a reault of which the animals died from botulism.

4. The botulism microbe possesses infectious properties and appears to be a pathogenic microbe.

Clinical observations and experiments on animals have established that the botulinic toxin proceeds from the alimentary canal into the blood-carrying vessels, where it is discovered easily with the help of biological tests on animals. However to date the action of the toxin upon the blood-carrying vessels and the path for its penetration into the central nervous system have not been explained.

The experimental observation conducted by us decisively refutes the point of view that the botulinic toxin strikes mainly at the nervous system, not causing in this connection pathological transformations in other organs.

Our experiments prove beyond a doubt that the vessels of the animal organs and human organs react to the botulinic toxin by strong contraction, while at the same time the vessels of these same animals and human beings provide an insignificant reaction to toxin that has been dispersed through boiling.

In such a way, the results of tests support the conclusions of clinical workers about the vessel-contracting action of the botulinic toxin.

The hematogenous path as a means for distribution of the toxin throughout the whole organism, apart from the central nervous system, exists without any doubt. The path for the penetration of the botulinic toxin into the central nervous system has not been explained to the present time.

The following has been established, on the basis of tests conducted with animals which received intravenous injections of large quantities of botulinic toxin:

1. The absence of toxin from the spinal cord fluid while it is present in large quantities in the blood even after the death of the animal. This fact appears to be unchallenged proof that the toxin penetrates into the central nervous system along lymphatic paths, since the latter have been reported with the sub-membrane area of the brain (Speranski, 1935).

2. The penetration of the botulinic toxin by hematogenous means (through destruction of the capillary walls and those of the

pre-capillaries) into the central nervous system, where it adsorbs itself quite strongly; during subcutaneous injections of the brain suspension into animals, it does not manifest its poisonous property.

The data obtained by us in experimental research on the pathogenesis of the botulinic intoxication may be reduced to the following:

1. The toxin penetrates from the alimentary canal into the blood-carrying vessels.

2. The toxin spreads over the whole organism with the blood.

3. Situated in the blood, the toxin causes a strong contraction on the part of the vessels, paralysis of the miocardia and walls of the vessels.

4. The toxin penetrates through the damaged walls of blood-carrying vessels into various organs as well as into the central nervous system.

In animals infected with doses of spores from the botulism microbe, there develops a symptomless infection which may last over a long period of time and be accompanied by a very slow multiplication of microbes in the organs.

Under the influence of toxic products of microbe viability, the immunological condition of cells in the smooth musculature of vessels is transformed. As a result, there takes place the formation of agglutinins (at times in insignificant quantities - of anti-toxin) and a weakening of the reaction by vessel cells upon the toxin. In such a way, the symptomless infection caused by spores leads to the development of immunity by the cells of the smooth musculature in vessles.

Experimental observations in order to explain the mechanism of immunity during botulism permits the following conclusions:

1. During immunization with the botulinic anatoxin, there occurs in animals a deep transformation not only in the blood but also in the cells of the organ tissue: a cellular and humoral immunity toward the botulinic toxin develops.

2. The anti-toxin immunity during botulism, as also in dyptheria and tetanus, is conditioned by the presence of anti-bodies and the areactiveness of tissue cells in the organism toward toxin.

3. During contamination with spores, the immunity of cells in organs develops propitiously thanks to immunization from the toxin which is formed in the organism. Infection with spores and immunization by the anatoxin identically cause the areactiveness of cells in the organs of animals toward the botulinic toxin. In this and in other cases, immunity is precipitated by one anti-gene; this appears to be proof about the production of toxin in the organism during the contamination of animals with sub-lethal doses of spores.

4. The obtained results confirm and support the necessity for applying anatoxin during the treatment of botulism.

5. Better results are obtained during the serum therapy for botulism with a single immunization by anatoxin, especially in hard cases of the disease. The latter depends upon changes in the condition of tissue cells within the patient's organism; these assume areactiveness toward the toxin produced by microbes.

By studying the areactiveness of the central nervous system during serum therapy and during active immunization, it was established that in the serum therapy against botulism and tetanus the anti-toxin practically does not penetrate into the liquiform cystern. However this fact can not serve as the basis for claiming the

defenselessness of the central nervous system during serum therapy and during active immunization against toxinemic infections. Our experimental data indicate an areactive condition in the central nervous system during passive and active immunization against botulism and tetanus. This shows that the anti-toxin situated in the blood penetrates through the capillary walls into the intramural liquiform, directly washing the brain cells. The toxin located in direct contact with the nervous system neutralizes itself by means of the anti-toxin.

Practical conclusions from our research are contained in the following: during serum therapy for botulism and tetanus, it is necessary to inject as soon as possible intravenously large quantities of serum; simultaneously, the serum should also be injected in large quantities intra-muscularly for the purpose of maintaining a high titer of the anti-toxin in the blood of the patient.

Studying the formation of agglutinins in laboratory, domestic animals and in healthy human beings showed that in the pathogenesis of botulism a considerable role is played by anti-bacterial immunity. In animals, infected subcutaneously or per mouth with the spores of the botulism bacillus, there is observed the formation of agglutinins in large quantity. At the same time, and absence of anti-toxin from their blood is observed; on the other hand immunity of the organ cells toward the toxin is present.

This phenomenon can be explained by the fact that the microbe produces an insufficient amount of toxin to irritate the cells participating in the production of anti-toxin; this is corroborated by the absence of it from the blood of animals during many injections

of small botulinic toxin doses into them in sequence. After conducting an agglutination reaction with the microbe of botulism and serum from pigs and cattle, it appears to be positive in those animals in a large percentage of cases. The presence of agglutinins in a high titer throughout the blood of certain animals may be explained by the fact that they were infected with spores while eating fodder.

Studying the reaction of agglutination with the botulinic bacillus and human serums, it appeared that in a part of the cases the reaction was positive when the serum was highly diluted (from 1:800 to 1:1600), in others when it was low, but finally in the majority of cases it was negative.

These data definitively point toward the development of a symptomless botulinic infection in certain persons; this infection precipitates immunological transformations in the organism.

The stability of individual persons to the toxin apparently can be explained by the development in them of an immunity when they consumed with their food products that were infected by the spores of botulism.

So, the human being and animals, when consuming food of an inferior quality at times become infected by the spores from the microbe of botulism; as a result of this, they develop an anti-bacterial and anti-toxic immunity.

The botulism bacillus appears to be the most promising model for the study of the pathogenesis of other symptomless anaerobic infections. During a symptomless infection caused by the spores of an anaerobe, there takes place an endless process of interaction between the microbe and the macro-organism. A hypothesis to the effect that

the spores of anaerobes can remain for a certain time in a non-active condition, does not correspond with reality.

The microbe, having penetrated into the organism, immediately begins to manifest its viability and causes immunological transformations in the organism. The microbe and the macro-organism during the whole time of the infection manifest endless action one upon the other; as a result of this, the infection is destroyed and immunity develops or the disease commences. The final outcome depends upon the aggressiveness of the infectious agent and the defensive capabilities of the macro-organism.

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