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The application of the complement-fixation reaction as a means of directing minute quantities of decomposition of beef

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THE APPLICATION OF THE COMPLEMENT-FIXATION REACTION AS A MEANS OF DETECTING MINUTE QUANTITIES OF DECOMPOSITION OF BEEF

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INTRODUCTION

Meat in the diet of mankind is important, and it is of interest to the public that it be wholesome and free from any products which might cause disturbances in human alimentation and digestion. The organic nature of meat makes it a suitable medium for the development of aerobic and anaerobic bacteria. From the time the animal is slaughtered until it reaches the consumer, unless most rigid methods of refrigeration are practiced there are opportunities for contamination.

The condemnation of meats by sanitarians rests upon uncertain foundations. The organoleptic test, a common method used which consists of testing by the senses of sight, smell, feel and perhaps taste, has been satisfactory for general purposes. Often, however, a more accurate test is desired. For these reasons it is important that some rapid test be available which can be applied to determine whether or not meat has been handled under sanitary conditions, and to determine whether the meat is strictly fresh or whether it is in the beginning stages of putrefaction.

Immunological reactions were originally studied with the purpose of solving urgent problems concerning the cure, diagnosis and prevention of disease. Their importance as a general biological phenomenon not exclusively concerned with disease is now recognized. Precipitin and complement-fixation tests are used for detecting and identifying proteins.

These facts have stimulated the writer to study methods for detecting minute quantities of decomposition in beef by complement-fixation tests and modifications of the technique as applied to diagnostic and differential procedures.

HISTORICAL

In 1898 by using the serum of a guinea pig immunized against the corpuscles of a rabbit, Bordet[1] was able to demonstrate the phenomenon of serum hemolysis. He described the two substances present in the serum concerned in the procedure, the specific substance the antibody and the non-specific substance the complement.

In 1901 Bordet and Gengou[2] conducted the first complement-fixation test, using antipest serum for their specific amboceptor, and a suspension of Bacillus pestis for an antigen.

The same year Widal and Lesourd[3] made practical application of the Bordet-Gengou phenomenon. They applied it to the serum diagnosis of typhoid and tuberculosis, but with little success.

Wassermann, Neisser and Bruck[4] in 1906 published results on investigations conducted on complement-fixation in the serum diagnosis of syphilis in monkeys. After this work the reaction was established as a laboratory method.

Since this first work on complement-fixation numerous applications have been made of the reaction, not only in the diagnosis of disease but also for identifying and differentiating proteins and bacteria. Within the last few years it has been applied to identifying specific animal proteins in foods, as detecting the presence of cat, horse or dog meat in beef.

Because of the sensitiveness of the complement-fixation reaction it has come into practical use for identifying unknown antigens. Hektoen, Carlson, and Schulhof[5] have demonstrated the sensitiveness of immunological reactions by their work on thyroglobulin. They have shown that the

presence of thyroglobulin in the lymph from the thyroid in amounts far too small to be detected by any analytical or physiological methods may be demonstrated by immunological reactions.

Koessler and Koessler[6] also have demonstrated the delicacy of complement-fixation reactions by using it to show the existence of a specific scarlatinal virus in the tissues during this disease, although the actual organism had not been isolated.

Complement-fixation tests have been found applicable in the analysis of blood stains, also in detecting the presence of adulterants in meats and milk. Kolmer[7] has applied it for detecting meat adulterations as the presence of cat, horse or dog meat in sausages or hamburger, also in the identification of milks, although it is lacking in sufficient specificity for detection of goats' milk added to cows' milk.

In 1923 Kesler[8] applied the complement-fixation reaction to the identification of Clostridium botulinus and its toxin in canned food stuffs. He used botulinus antitoxin as his immune serum and a saline extract of the infected food as his antigen. He asserts that he was able to differentiate the biochemical changes produced by Clostridium botulinus from those of other organisms, but the procedure did not give type differentiation.

Gail[9] in 1926 also applied the test as a method for the laboratory diagnosis of botulinus, but found it not entirely successful. He found that antigens from the supernatant centrifugalized material of vegetables which were artificially inoculated with Clostridium botulinus had a high anticomplementary value, and the ratio between their antigenic and anticomplementary values was found to be small.

Complement-fixation tests for the sanitary examination of foods have been applied only to the extent mentioned. Other biological, chemical and physical means have been used especially in the examination of milk and meat.

Bacteriological methods for meat analysis have been much used. Marxer[10] has set a standard on the bacterial count for meat as 1,000,000 per gram. Weinzirl and Newton[11], however, made an analysis of forty-four samples of market hamburger steak and found that all but four exceeded 1,000,000. So, according to Marxer's standard, practically all hamburger would be condemned. On the basis of the organoleptic test practically all samples would pass. In 1914, these same authors [12], using a standard technique which they devised, arrived at a standard of 10,000,000 bacteria per gram as a limit for condemning meat.

Carey's[13] studies on bacterial counts and on their significance to sanitary quality of meat show that the number of bacteria per gram of sausage varies so widely that little importance can be attached to the bacterial count alone. Many factors, such as precautions used in manufacture, proper handling, and the presence of preservatives, may influence the count.

Savage[14] also believes that little importance can be attached to the number of bacteria present, but considers the presence of intestinal bacteria of greater significance.

Brewer[15] working with various cuts as obtained from meat markets found quite a variation in counts. From his investigations, the Marxer and Weinzirl standards would both be too low. As an average on hamburger steak, he obtained a count of 46,800,000 which included colon bacteria, putrefactive bacteria, liquifiers and cocci.

Hoffstadt[16] using ground beef attempted to associate the bacterial count with the sanitary conditions of shops and with the organoleptic test. She concluded from her work that the bacterial count is an unreliable standard for meat analysis.

The presence of products of proteolysis which can be detected by chemical means has been used with some success as an index for sanitary analysis of meat. Ottolenghi[17] found that the determination of amino acids was valuable as an index of the beginning of active decomposition.

Bokman[18] used the same method in examining fish with equally good results.

On the other hand Sears[19] found that peptone cultures of most bacteria give fluctuating concentrations of amino acids, showing that these bodies are formed and broken down continuously by the organisms. A few proteolytic organisms show increasing concentrations. He obtained the same results with ammonia. He concluded that neither alone was a good index of decomposition.

Ammonia tests have met with more approval than the amino acid tests, because ammonia is an end product, while amino acids are intermediate products.

In 1894 Eber[20] proposed a qualitative test for ammonia as a test for beginning putrefaction, but the test proved to be of little value as it proved to be an indicator of advanced putrefaction instead of beginning putrefaction.

Falk, Bowmann and McGuire[21] made determinations on total nitrogen, non-protein nitrogen, ammonia nitrogen, total creatinine nitrogen and purine nitrogen produced by organisms thought to be responsible for actual cases of food poisoning. They found the ammonia nitrogen was the only one which consistently increased with spoilage.

Falk and McGuire[22] found that ammonia results obtained from meat spoilage depended on the temperature at which the meat was kept. Meat stored at room temperature was found to be unsuitable for food when the ammonia nitrogen reached 0.3 to 0.4 mgs. per gram of meat; here the bacterial count was high. When meat was kept at a low temperature the ammonia was found to be much higher before the meat became unsuitable to eat; the ammonia nitrogen reached 1.0 - 3.0 mgs. before it became unfit for a food. With this the bacterial count was low.

From the literature cited it is evident that there is much controversy on the reliability of the methods now at hand used in the sanitary analysis of meat. A method which would directly indicate putrefaction in its early stages is desirable.

Since by immunological methods it is possible to stimulate antibody formation with resultant specificity for antigen used, the possibility was suggested to the writer of using serological methods for detecting putrefactive substances in beef.

THEORETICAL CONSIDERATIONS

From the standpoint of serum diagnosis complement-fixation may be used in one of two ways: if the antigen is known the antibody may be detected, or if the antibody is known the antigen may be detected. Under the first example may be classified the Wassermann reaction where an extract is employed as antigen which is known to have the property of interacting with syphilis antibodies if present in the serum. Complement-fixation reactions for typhoid, glanders, gonorrhea, etc., where an antigen of the specific bacteria is used for the detection of specific antibodies, are also reactions of the first type. Under the second type of reactions we have the reverse of the above; a serum of known antibody content is employed to detect an unknown antigen. Complement-fixation tests of the second type are employed in identifying and differentiating proteins and bacteria.

This reaction permits us to determine infinitesimal amounts of proteins concealed in mixtures of great complexity and to determine facts about these proteins that cannot be determined by any chemical means.

Because of the delicacy of the reaction the author conceived the idea that the reaction would be of value in determining the presence of minute quantities of decomposition in beef. Any changes in the protein of the meat brought about by autolysis or by bacterial action, although small, and of such nature as not to be detected by chemical means, may be demonstrated by complement-fixation reactions, by immunizing a susceptible animal and recovering the specific antibody.

Only antigens which are of protein nature are believed to be capable of stimulating an animal to produce antibodies. The antigenicity of a substance may be determined by injecting it into the body of an animal in definite quantities and under suitable conditions, to see if it leads to the production of antibodies demonstrable in the blood by the reactions they exhibit in the presence of the specific antigen. The assumption is made that the development of these antibodies is a defense against the presence of the protein. The protein needs to be foreign in nature.

Not all tissues of the body are permeable to foreign proteins; the epithelium of alimentary and cutaneous surfaces exhibit a protective action against them. Hydrolysis of proteins in the digestive tract destroys their antigenic properties, so cleavage products which pass the intestinal walls do not stimulate antibody formation. Wells[23] suggests that "an important and perhaps essential factor in the antigenic activity of proteins is their large molecular dimensions with attendant colloidal properties. Whenever the protein molecule is broken down into smaller fragments it loses its antigenic properties pari passu."

Zinsser suggests that antibodies are formed in the animal body only upon injection of entirely non-diffusible substances like the true proteins. True non-diffusible colloids are unable to enter the cells where they would be destroyed, so extracellular substances or antibodies are formed and given off into the blood stream. These may react with the foreign protein. Any complete soluble protein would therefore be antigenic.

Gelatin which is not a complete protein exhibits no demonstrable antigenic effect. It is believed that the lack of antigenic property is due to lack of the amino acids containing the aromatic radicles for it does not contain any tryptophane or tyrosin and but a small amount of phenylalanine. Obermayer and Pick[24] have found that it is the aromatic radicle of the protein molecule which is of importance in determining the specificity of an immunological reaction. Of the toxic and non-toxic fractions of protein cleavage, the toxic fraction contains the aromatic radicles, and it is this fraction which is the determining factor in the stimulation of antibody formation.

In this investigation it is assumed that the saline extract of decomposition products of beef used as the inoculum in the immunization of susceptible animals for acquiring antibodies to be used as a laboratory reagent, contains toxic cleavage products of the protein produced by bacterial action and by autolysis of the meat.

Wells and Osborne[25] have shown that the antigenicity of vegetable proteins indicates that the antigenic activity depends on the solubility of the antigen in the tissue fluids. This, however, cannot be said of all proteins; the amount and rapidity of antibody formation varies much with different animals. The amount of antibody formation does not vary directly with the amount of antigen injected, small doses often producing even larger amounts of antibodies than large doses. Animals also show individual variations in antibody production in response to the same amount of antigen. This variation is found even in animals of the same species.

What has been said of antigen applies to antigens "in vivo" but not necessarily to antigens "in vitro". Antigens have two functions, one to react in the animal body to produce antibody formation, and the other is the capacity to react with antibodies in the test tube, and these two properties are not always identical. An example is the Wassermann reaction for serum diagnosis of human syphilis in which lipoids which are not capable of inciting antibody formation are capable of reacting with the antibodies in the complement-fixation test.

The explanation given for the inability of lipoids to stimulate antibody formation in the animal body is that lipoids from different animals, even of different species, are chemically the same. Levene(26) has found that there is no distinction between lipoids derived from different tissues or different species. However, a lipoid united with a protein will give a different immunological reaction than the protein alone will give. An example is the different reactions produced by euglobulin and pseudoglobulin, the difference in the two chemically being the presence of a lipoid group in the euglobulin.

The addition of various non-protein radicles to protein antigens may alter their specificity. Proteins combined with various organic compounds act as antigens which produce antibodies reacting with any sort of protein to which the same or similar chemical groups are attached.

Immunological differences between proteins are usually dependent upon chemical differences which can be detected by chemical or physical means. It is believed that specificity is not dependent on the

entire protein molecule but that there are certain groups or radicles of the protein molecule which are responsible for specificity, and that a single protein molecule may contain two or more such groups. This specificity may be altered by changing physically or chemically these groups.

Group reactions and non-specific immunization may be accounted for by the fact that as lipoids, fats and carbohydrates in different species are chemically identical so may tissue proteins in different species or animal cells be identical.

Eberson[27] has obtained evidence showing that group reactions of different bacteria of the same species is due to a common antigenic protein. Group reactions, however, may be largely eliminated by titrations of the antiserums.

As yet specificity of these biological reactions has not been explained either by physical or chemical means. There is no chemical method by which one variety of protein can be distinguished from another, yet the serum antibodies of one species of bacteria reacts only with that species. Hemolysins, agglutinins, or precipitins react respectively with the antigen used in producing them. Wells believes that this indicates that each of the antigens possesses an individual chemical structure responsible for the specificity of the reaction, chemical methods indicating only proteins in general.

We have no knowledge as to what these antibodies are; like enzymes they are known only through their behavior. Precipitins, agglutinins, cytolysins, antitoxins, etc., have each been accepted as distinct types of antibodies because of the distinct reactions produced by each.

More recent investigations, however, tend to show that these antibodies are all of one type which react differently according to the procedure used to designate them.

Dicks[28] has arrived at the hypothesis that there are two fundamental types of immunity reactions,"one having to do with substances which are essentially active poisons, antibodies neutralizing or inhibiting their toxicity by direct chemical action. In this group come the antitoxins, antibodies for venoms, vegetable toxins, and bacterial hematotoxins. It is to be noted that the toxic substances, the true toxins, are all similar to one another in being classed as large colloidal aggregates resembling proteins but not identified as proteins.

The other group of immunity reactions is concerned with defense against foreign proteins whether toxic or non-toxic, whether in solution or aggregated into cells. In all the reactions of this group we deal with processes that tend to alter the colloidal state of the foreign proteins by making them larger aggregates (precipitation and agglutination) or smaller aggregates (proteolysis, hemolysis, bacteriolysis and cytolysis) and in each case the reaction consists of two separable steps, sensitization and reaction."

In this project concerning decomposition of beef the immunity reactions concerned are of this group, and the antibody formation is in defense against the cleavage products of the protein introduced. Theoretically, there would be antibodies formed which would produce precipitin and agglutination reactions along with the proteolysis.

In view of the "unitarian" hypothesis presented by Zinsser[29], immunity is explained as the result of simple physico-chemical conditions between the colloids of the serum and the antigens. "There is but one kind of antibody which has the property of rendering an antigen susceptible to coagulation by the electrolytes present, causing precipitin or agglutination reactions. If complement is present the lytic phenomenon results giving hemolysis, cytolysis or bacteriolysis." This idea has been advanced by many investigators but because of the lack of quantitative parallelism between the curves of antibody functions in the same serum it has not been given much consideration.

The identity of agglutinins and precipitins has raised little question as both are specific flocculation reactions between serum and the same antigenic substance, depending upon analogous environmental conditions. The difference in the flocculation is due to the antigen; in one case the antigen is present in relatively large masses and in the other it is finely dispersed.

Our present knowledge is limited to knowing antibodies only from their reactions. This investigation is concerned chiefly with that phase of immunology which deals with complement-fixation reactions, so the complement-fixing bodies and the nature of complement will be considered.

Bacteriolysis, hemolysis, proteolysis and cytolysis depend upon the presence of two agents, the antibody or amboceptor which sensitizes the foreign substance present and the complement, a component of normal animal serum, which unites with the sensitized substance. The reaction is shown in the test tube by the union of the complement with sensitized

antigen. The function of the diagnostic amboceptor is to sensitize the antigen or give it an affinity for complement.

To demonstrate the action of complement an analogy may be drawn between the actions of insulin and antibodies, since each appears not to be the active agent itself, but merely one that accelerates or makes possible the action of another substance. The amboceptor serves to activate the complement bringing about lytic reactions and the insulin makes possible the burning of sugar in the tissues, neither in itself accomplishing the final reactions.

The complement then may be considered as the active agent. If a serum containing antibacterial antibodies is heated for fifteen minutes at 55° C. it is found to lose its power of destroying bacteria. Unheated normal sera is equally without effect. If, however, to the heated immune sera is added some unheated normal sera the mixture will be found to be actively bacteriolytic as the original unheated serum. This indicates that aside from the antibodies present there must be a second substance, each in itself incapable of destroying bacteria, but one dependent on the other. The second substance present or the complement is considered to be non-specific, the specificity of the reaction depending on the antibody. The antigen by union with its specific antibody is sensitized to the action of the complement and the complement is the substance that actually destroys the cell.

A peculiar phenomenon of non-specific reactions has been observed in complement-fixation reactions. Normal rabbit, dog and mule sera have been found to yield falsely positive or non-specific reactions when used with antigens, both of lipoidal or protein nature. There has been little explanation offered as to what causes these reactions but

investigators have shown something of their nature and how they may be eliminated.

This problem of non-specific reactions has had to be overcome when using animals for producing immune sera for the purpose of identifying specific unknown antigens. This subject possessee much interest both from an academic and practical standpoint as rabbits are commonly employed in experimental syphilis and their sera used in Wassermann tests; also rabbit sera are commonly used in complement-fixation tests for identifying and differentiating proteins and bacteria.

Kolmer and Trist(30) studied this non-specific complement-fixation reaction produced by normal rabbit sera. With the normal sera they used bacterial antigens and lipoidal extracts used in the Wassermann test. Normal rabbit serum was used according to the usual technique, in active condition and after inactivating at 55° C. for one-half hour. They found that non-specific complement absorption occurred in the presence of lipoidal antigens and bacterial antigens. Kolmer, Trist and Heist(31) obtained results on normal dog sera comparable to those observed on normal rabbit sera. These investigators attribute such results to be probably due to the presence of native hemolytic complement and antisheep hemolysin present in the sera.

A study was made by Mackie and Finkelstein(32) on non-specific reactions with reference to the interaction of normal serum and certain non-antigenic substances. A large number of varied agents were tested mainly with a view to ascertain whether their "pseudo antigens" belong to any particular chemical group. Commercial peptone, various alcohols, amino acids and cholesterolized alcoholic tissue extracts used in the Wassermann reaction were tested. All were capable of giving

positive reactions. Of the amino acids glycine yielded the most pronounced reaction.

On this present investigation the author found that normal rabbit sera yielded positive reactions with extracts from decomposed meat while not with extracts of fresh meat. It may be that these falsely positive reactions were due to the presence of amino acids present in the extract of decomposed beef, the fresh meat extract giving negative results as no proteolysis had taken place in it.

Kolmer[33] attributes the non-specific complement-fixation reactions obtained with normal rabbit and dog sera to be due to both the serum lipoids and proteins present in the serum. By extracting sera with ether and chloroform the antilytic and complement-fixing powers were diminished indicating that the serum lipoids were responsible for these processes, whereas the enteral and parenteral administration of lipoids increased the antilytic and complement-fixing powers. He also observed that the globulin and albumin fractions of normal rabbit and dog sera possess thermostabil antilytic and complement-fixing properties, usually the former to a slightly greater degree.

Noguchi[34] succeeded in extracting with ether an antilytic principle from normal sera called protectin that was found capable of withstanding temperatures as high as 90° C. and capable of inhibiting serum hemolysis directly by neutralizing complement and indirectly after absorption by corpuscles by increasing their resistance.

Kolmer and Trist[35], also Mackie and Finkelstein, have found that these antilytic and complement absorbing substances present in

normal sera are thermolabile. Tendencies for non-specific reactions are much decreased when sera is heated to 62° C. for 30 minutes and entirely removed by heating to 70° C. for 30 minutes.

Results obtained by the author confirm with those of Kolmer and Trist, and Mackie and Finkelstein.

The experimental evidence obtained in this problem indicates that there is something present in an extract of decomposed beef which has the property of stimulating antibody formation in susceptible animals. It is assumed that it is protein in nature and that it is present only in small amounts and variable, as the antibody content of the serum of immunized animals was low.

EXPERIMENTAL

The object of this experiment, as first planned, was to determine, through the use of immunological reagents, a method which would reveal specific types of decomposition in beef. The decomposition products of Clostridium botulinus, Type A, were selected because of the slight evidence of putrefaction produced by this organism when present in food, and because of its importance in food poisoning.

The object was to produce a sufficiently sensitive immune amboceptor which would sensitize a meat extract antigen containing the decomposition products of Clostridium botulinus, thus establishing a specific antigen-antibody complex where a fixation of complement would take place. The immune serum, however, proved to be non-specific for the putrefactive substances produced by Clostridium botulinus but gave a fixation of complement with other types of putrefaction. However, fresh cooked beef or that in which no decomposition had occurred yielded negative results. As negative reactions were produced with strictly fresh meat a continuance of the project seemed justifiable, as it indicated that by complement-fixation fresh meat could be differentiated from that which was beginning to decompose. This fact led the author to prepare an immune serum against the natural decomposition products of raw beef which could be employed to differentiate fresh beef from that which had decomposition products present, regardless of their source.

The experimental procedure used in preparing an antiserum against the decomposition products of Clostridium botulinus will be described. Although this method as followed out did not yield entirely satisfactory

results, it did indicate, as stated before, that by complement-fixation reactions fresh meat could be distinguished from meat which had decomposition products present even though present in small quantities.

The Preparation of the Immune Amboceptor Against the Decomposition Products of Clostridium botulinus:

Preparation of antigen used as the inoculum for the immunization of susceptible animals:

Fresh lean beef was ground to the consistency of hamburger steak, 10-gram portions were weighed and placed in large test tubes, 21.5 cm. x 2.5 cm.; 25 cc. of physiological saline were added to each tube and the contents thoroughly mixed. The tubes were then stoppered and sterilized in the autoclave for 20 minutes at 15 pounds pressure. As needed the tubes were inoculated with two loops of a pure culture of Clostridium botulinus, Type A, and incubated at 37° C. for 72 hours. The depth of the media allowed for anaerobic conditions. To ascertain the purity of the culture of Clostridium botulinus it was examined both microscopically and through its biochemical reactions on dextrose agar, litmus milk and potato slants. It was found to be morphologically typical and it gave the characteristic culture reactions.

For the first six inoculations the toxin, produced in the meat media by the growth of Clostridium botulinus, was destroyed by heating the meat for one-half hour at 80° C. As the object of the experiment was to produce an antiserum against the decomposition products of Clostridium botulinus and not the bacillus itself, the meat media

which had been thoroughly mixed was filtered through a Seitz filter so as to remove all the bacteria and spores. The clear filtrate was used as the inoculum.

Immunization of the Animals:

Six healthy rabbits ranging in weight from 2150 to 3300 grams were used for producing the antiserum. The inoculations were made intravenously as less antigen is required than if done intraperitoneally and there is less danger of infection. Intraperitoneal injections are much more likely to give a high death rate because of peritonitis. Injections were made in the posterior auricular vein along the outer margin of the ear, commencing as near the tip of the ear as possible and subsequent injections were made nearer and nearer the root. Inoculations were made every other day with amounts ranging from 0.5 cc. to 2 cc. Three inoculations were given with the 2 cc. quantities; the total number given was six.

The animals were allowed to rest for five days then blood was drawn from the marginal vein of the ear and a preliminary test was made on the serum for its antibody content.

The serum was found to be low in antibody content so injections were continued using unheated filtrates containing the botulinus toxin. To ensure against death of the rabbits because of the toxin present, the antigen was titrated for its toxicity.

Titration of the Antigen for its Toxicity:

Six guinea pigs weighing approximately 200 grams each were inoculated subcutaneously with 1 cc. of the unheated filtrate containing

the botulinus toxin. The first five pigs were injected subcutaneously with botulinus antitoxin Type A, with amounts ranging from 50 units to 375 units; the sixth did not receive any antitoxin.

The sixth pig and the one receiving only 50 units of the antitoxin died within 48 hours, showing the typical symptoms of botulinus poisoning. This gave only an approximate idea of the toxicity of the antigen, but enough for the purpose in this experiment as the object was to ascertain how much antitoxin would be necessary to protect the rabbits.

Immunization was continued in the rabbits using the unheated extract as the inoculum. With the first inoculation of 1 cc. of the antigen, 750 units of the antitoxin were given. Three subsequent inoculations of the antigen were given every other day, in amounts of 1.5, 2 and 2 cc. The rabbits were allowed to rest for five days and were then bled from the marginal vein of the ear; the serum was again tested for antibody content.

Preparation of the Antigen to be Used in the Diagnostic System Against an Established Hemolytic Complex:

The antigen for the experimental test was prepared in the same manner as the antigen used in immunizing the rabbits, except that the time for incubation was varied, and inoculations were made not only with Clostridium botulinus Type A but also with Clostridium botulinus Type B, Clostridium sporogenes, Proteus vulgaris and Pseudomonas fluorescens.

Guinea pig complement was used in conducting the experiments. Since the sera from the individual guinea pigs vary in the content

of complement, a mixture of sera from two or three guinea pigs pooled together was used. The complement was obtained by bleeding the animals from the heart as required.

A two per cent suspension of washed red blood corpuscles obtained from a sheep was employed in the hemolytic system. An anti-sheep hemolysin obtained from immunized rabbits was used to sensitize the red blood corpuscles. A hemolysin having a high titre was chosen to eliminate any chances of non-specific reactions.

Procedure for the Test; Titrations:

As suggested by Kolmer(36) the hemolysin and complement were both titrated before each day's work. He recommends the practice of daily titration of the hemolysin in order to adjust for any natural antisheep hemolysin apt to be present in the complement serum. Under these conditions it is unnecessary to exclude complement serum containing natural hemolysin or to remove the hemolysin beforehand.

Titration of Hemolysin:

0.5 cc. of varying dilutions of hemolysin were placed respectively in a series of 10 standard serological test tubes (85 x 15 mm.). These standard tubes were used throughout the experimental work as results are more easily interpreted if tubes are of a regulation size.

The range of dilutions varied with the ambeceptor; a range from 600 to 4,000 was that usually employed in this experiment.

0.3 cc. of a 1 to.10 complement (dilution of the complement used depends on the freshness of the complement) were added; to this 0.5 cc. of the 2 per cent suspension of washed red corpuscles and 1.7 cc. of saline were added, making the total volume of each tube 3 cc.

The contents of each tube were mixed and placed in a water bath at 38° C. for one hour.

The hemolytic unit was determined as that mixture containing 0.5 cc. of hemolysis of the highest dilution just showing complete and sparkling hemolysis of 0.5 cc. of the 2 per cent suspension of washed red blood cells of the sheep.

For the test a dilution was used in which 0.5 cc. contained two full units.

An 11th tube was used as a control on the hemolysis.

TABLE 1

Titration of Hemolysin

Tube	Hemolysin	Complement 1 to 10	Corpuscles 2 per cent	Saline	After water bath incubation for one hour
1	0.5 cc. 1:600	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
2	0.5 cc. 1:700	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
3	0.5 cc. 1:800	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
4	0.5 cc. 1:900	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
5	0.5 cc. 1:1000	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
6	0.5 cc. 1:2000	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
7	0.5 cc. 1:2500	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
8	0.5 cc. 1:3000	0.3 cc.	0.5 cc.	1.7 cc.	Complete hemolysis; unit
9	0.5 cc. 1:3500	0.3 cc.	0.5 cc.	1.7 cc.	Marked hemolysis
10	0.5 cc. 1:4000	0.3 cc.	0.5 cc.	1.7 cc.	Slight hemolysis
11	0.5 cc. 1:600	None	0.5 cc.	1.7 cc.	No hemolysis

In the above titration the unit was recorded as 0.5 cc. of the 1 to 3000 dilution of the hemolysin; 2 units would be contained in 0.5 cc. of a 1 to 1500 dilution.

Titration of the Complement:

In a series of 10 test tubes were placed respectively, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 cc. of the 1 to 10 complement; the tenth tube served as a corpuscle control.

To each tube the following were added: 0.5 cc. of the hemolysin containing 2 units, 0.5 cc. of the 2 per cent corpuscle suspension, and enough saline to make the total volume 3 cc.

The contents of each tube were mixed and placed in the water bath at 38° C. for 1 hour.

The unit of complement was determined as the smallest amount of the complement diluted 1 to 10 which would bring about complete hemolysis in the presence of the other standard components of the hemolytic system.

The full unit was recorded as that amount of complement representing the next higher increase of complement in the titration series.

Two full units of complement diluted with sufficient saline to make 1 cc. (Kolmer[36]) were used in the test.

In the following titration the full unit was recorded as 0.3 cc. of the 1 to 10 complement; the working dose used would be 0.6 cc. This quantity of complement is used in 1 cc. volumes. These volumes are obtained by diluting the 0.6 cc. dose of complement to 1 cc.; the dilution is divided by the dose. This gives a dilution 1 cc. volume of which contains 2 full units or the working dose.

TABLE II

Complement Titration

Tube	Complement 1 to 10	Hemolysin (2 units)	Corpuscles 2 per cent	Saline	After water bath incubation for one hour
1	0.1 cc.	0.5 cc.	0.5 cc.	1.9 cc.	No hemolysis
2	0.15 cc.	0.5 cc.	0.5 cc.	1.9 cc.	Slight hemolysis
3	0.2 cc.	0.5 cc.	0.5 cc.	1.8 cc.	Marked hemolysis
4	0.25 cc.	0.5 cc.	0.5 cc.	1.8 cc.	Complete hemolysis(exact unit)
5	0.3 cc.	0.5 cc.	0.5 cc.	1.7 cc.	Complete hemolysis (full unit)
6	0.35 cc.	0.5 cc.	0.5 cc.	1.7 cc.	Complete hemolysis
7	0.4 cc.	0.5 cc.	0.5 cc.	1.6 cc.	Complete hemolysis
8	0.45 cc.	0.5 cc.	0.5 cc.	1.6 cc.	Complete hemolysis
9	0.5 cc.	0.5 cc.	0.5 cc.	1.5 cc.	Complete hemolysis
10	None	0.5 cc.	0.5 cc.	2.0 cc.	No hemolysis (Control)

The complete hemolytic system is thus established and standardized ready to be used as a laboratory reagent.

Titration of the Differential Antigen: Titration for Hemolytic Properties:

The following dilutions of antigen were prepared: 1:4, 1:6, 1:8, 1:10, 1:12, 1:16, 1:20 and 1:32.

0.5 cc. of the above dilution of antigen were placed respectively in a series of 8 test tubes.

0.5 cc. of the 2 per cent corpuscle suspension and 2 cc. of physiological saline were added.

The contents of each tube were mixed and incubated in a water bath for one hour at 38° C.

The smallest amount of antigen just beginning to produce hemolysis was considered as the hemolytic unit.

TABLE III

Hemolytic Titration of Antigen

Tube	Antigen 0.5 cc.	Saline	Corpuscles 2 per cent	After Water Bath for one Hour
1	1:4	2 cc.	0.5 cc.	No hemolysis
2	1:6	2 cc.	0.5 cc.	No hemolysis
3	1:8	2 cc.	0.5 cc.	No hemolysis
4	1:10	2 cc.	0.5 cc.	No hemolysis
5	1:12	2 cc.	0.5 cc.	No hemolysis
6	1:16	2 cc.	0.5 cc.	No hemolysis
7	1:20	2 cc.	0.5 cc.	No hemolysis
8	1:32	2 cc.	0.5 cc.	No hemolysis

Not any of the meat extract antigens used produced any hemolysis even in the lowest dilutions. This fact has been recorded in the above table.

Anticomplementary Titration of Differential Antigen:

In a second series of 8 tubes were placed 0.5 cc. of the above dilutions of antigen.

1 cc. of diluted complement (carrying 2 full units) and 0.5 cc. saline were added to each of the 8 tubes, also to a 9th tube (this to be used as a control on perfect balance of the hemolytic system.)

The contents of each tube were mixed and incubated in a water bath at 38° C. for 1 hour.

They were then removed from the bath and 0.5 cc. of hemolysin (2 units) and 0.5 cc. of the 2 per cent suspension of corpuscles were added to each tube; they were again mixed and re-incubated in the water bath for 1 hour at 38° C.

TABLE IV

Anticomplementary Titration of Antigen

Tube	Antigen 0.5 cc.	Saline	Complement (2 units)		Hemolysin (2 units)	Corpuscles 2 per cent	After Water Bath for 1 Hour
1	1:4	0.5 cc.	1.0 cc.	Water bath at 38° C. for one hour.	0.5 cc.	0.5 cc.	Slight inhibition of Hemolysis (unit)
2	1:6	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
3	1:8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
4	1:10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
5	1:12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
6	1:16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
7	1:20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
8	1:32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis
9	None	1.0 cc.	1.0 cc.		0.5 cc.	0.5 cc.	Complete hemolysis

The anticomplementary unit was recorded as the smallest amount of antigen inhibiting hemolysis in any degree.

The antigens prepared from cooked beef were found to be slightly anticomplementary.

Titration of the Immune Serum Produced in the Rabbits:

As the serum is the constant and the antigen the variable, titrations were made on the serums to determine their antibody content, thus determining the smallest fixing dose.

To avoid non-specific reactions the serums were heated one-half hour at 62° C.

In a series of 10 test tubes for each serum 0.5 cc. of the above dilutions of serum were placed.

An antigen of meat known to have some decomposition products present and therefore assumed to be antigenic was titrated for hemolytic and antioomplementary properties, and used in a dose of one-half of the anticomplementary unit. 0.5 cc. of the antigen were added to each tube and to an eleventh tube (antigen control).

These were allowed to stand five to ten minutes, then 1 cc. of the diluted complement carrying two full units was added to each tube, each tube was mixed and incubated in a water bath for one hour at 38° C.

The tubes were then removed from the water bath and the hemolytic system was added as described before. A twelfth tube was set up as a control on the hemolytic system. These were mixed and reincubated in the water bath for one hour at 38° C.

The smallest amount of immune sera which gave a + + + + reaction or that reaction which gave complete fixation of complement in the presence of the antigen referred to above was taken as the unit.

Only one unit of the sera was used in the tests as the sera dilutions used in their titrations were low.

In the procedure outlined it was found that it was necessary to inactivate the immune sera to 62° C. for 30 minutes as normal rabbit sera inactivated at 55° C. for 15 minutes was found to yield falsely positive reactions when used with an antigen of decomposed beef, while that heated at 62° C. for 30 minutes eliminated this non-specific property. By inactivating the immune sera at 62° C. any fixation which might occur would be due to the presence of specific antibodies and not to the substance present in normal sera which gives the falsely positive reactions.

Procedure used in testing normal sera of rabbits for nonspecific reactions produced when used with an antigen of decomposed beef:

The sera of eleven healthy normal rabbits was tested using both an antigen made of fresh meat and one from decomposed meat.

Sera inactivated at 55° C. for 15 minutes and also that inactivated at 62° C. for one-half hour were tested.

0.5 cc. of a 1 to 10 serum dilution were used as this amount was found to contain one unit in the immune sera.

TABLE 5

Titrations of Antisera

Tube	Antiserum 0.5 cc.	Antigen	Complement 2 full units		Hemolysin 2 units	Corpuscles 2 per cent		Readings
	Antiserum No. 126-127							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38° C. for 1 hour.	0.5 cc.	0.5 cc.	Water bath incubation at 38° C. for 1 hour.	+ + + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
	Antiserum No. 128-129							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38° C. for 1 hour	0.5 cc.	0.5 cc.	Water bath incubation at 38° C. for 1 hour.	+ + + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
	Antiserum No. 130-131							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38° C. for 1 hour	0.5 cc.	0.5 cc.	Water bath incubation at 38° C. for 1 hour.	+ + + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
	Antiserum No. 177-178							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38° C. for 1 hour	0.5 cc.	0.5 cc.	Water bath incubation at 38° C. for 1 hour.	+ + + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
	Antiserum No. 179-180							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38°C. 1 hr.	0.5 cc.	0.5 cc.	Water bath incubation at 38°C. 1 hr.	+ + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
	Antiserum No. 181-182							
1	1 to 5	0.5 cc.	1.0 cc.	Water bath incubation at 38° C. for 1 hour.	0.5 cc.	0.5 cc.	Water bath incubation at 38° C. for 1 hour.	+ + + +
2	1 to 8	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
3	1 to 10	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
4	1 to 12	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + + +
5	1 to 16	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
6	1 to 20	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ + +
7	1 to 32	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
8	1 to 40	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+ +
9	1 to 50	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		+
10	1 to 100	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-
11	1 to 5	0.5 cc.	1.0 cc.		0.5cc.	0.5 cc.		-
12	No Serum	0.5 cc.	1.0 cc.		0.5 cc.	0.5 cc.		-

In the following table will be found records of results obtained from the experiments which illustrate the effect of heat upon non-specific reactions produced by the normal sera of rabbits when used with an antigen of decomposed beef.

TABLE VI

Non-specific Reaction Produced by Normal Sera

Sera inactivated at 55° C. for 15 minutes					
0.5 cc. Normal Sera	0.5 cc. Antigen (Decomposed Beef)	0.5 cc. Antigen (Fresh Beef)	Serum Control	Antigen Control	Hemolytic System Control
Normal No. 1	+ + + +	-	-	-	-
Normal No. 2	-	-	-		
Normal No. 3	+ + +	-	-		
Normal No. 4	-	-	-		
Normal No. 5	+ + + +	-	-		
Normal No. 6	+ + + +	-	-		
Normal No. 7	+ + +	-	-		
Normal No. 8	+ +	-	-		
Normal No. 9	+ +	-	-		
Normal No. 10	+	-	-		
Normal No. 11	+ + +	-	-		
Sera inactivated at 62° C. for 30 minutes					
0.5 cc. Normal Sera	0.5 cc. Antigen (Decomposed Beef)	0.5 cc. Antigen (Fresh Beef)	Serum Control	Antigen Control	Hemolytic System Control
Normal No. 1	-	-	-	-	-
Normal No. 2	-	-	-		
Normal No. 3	-	-	-		
Normal No. 4	-	-	-		
Normal No. 5	-	-	-		
Normal No. 6	-	-	-		
Normal No. 7	-	-	-		
Normal No. 8	-	-	-		
Normal No. 9	-	-	-		
Normal No. 10	-	-	-		
Normal No. 11	-	-	-		

Quantitative Complement-fixation Test for Detecting the Presence of Decomposition Products of Clostridium botulinus Produced in Cooked Beef:

0.5 cc. of immune serum of a 1 to 10 dilution (amount found to contain one unit in the serums used), inactivated at 62° C. for one-half hour was placed in each of four test tubes for each of the antigens to be tested.

A dilution of the antigen in which 0.5 cc. contained one-half of its anticomplementary unit was prepared and 0.5 cc., 0.3 cc., 0.2 cc. and 0.1 cc. were added to each of the four tubes respectively. Sufficient saline was added to each to make the total volume up to 1 cc.

They were allowed to stand 5 to 10 minutes then 1 cc. of diluted complement carrying two full units was added. These were mixed and incubated in a water bath for one hour at 38° C. or in the Frigidaire at 10° C. for 16 to 18 hours so as to ensure fixation of complement if the reaction was positive.

After the primary incubation the hemolytic system was added; if incubated in the Frigidaire the tubes were warmed in the water bath for 10 minutes before adding the hemolytic system.

The contents of the tubes were mixed and reincubated in the water bath for 1 hour at 38° C.

Three controls were set up, one on the serum, one on the antigen, and one on the hemolytic system.

Reading and Recording the Reactions.

The method employed by Kolmer(37) in his quantitative complement-fixation test for syphilis was employed, adapting it to the varying amounts of antigen used, instead of varying amounts of serum.

Using the four amounts of antigen the degree of positiveness or inhibition of hemolysis was recorded for each amount as:

0.5 cc. of antigen = + + + +

0.3 cc. of antigen = + + +

0.2 cc. of antigen = + + +

0.1 cc. of antigen = +

The above reaction would be considered as strongly positive, the amount of decomposition present being high.

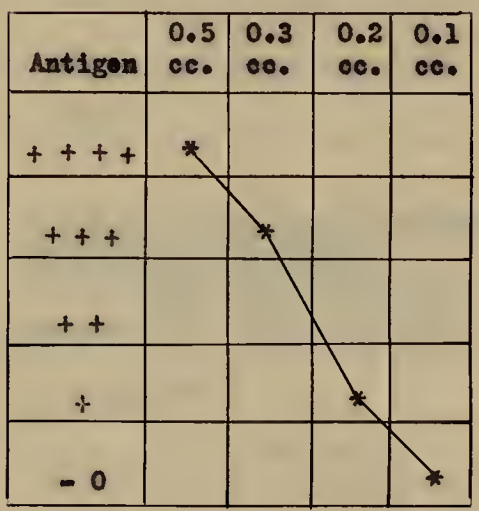
The following graphs are a record of the reactions produced when the various immune sera were used with meat extract antigens having present decomposition products produced by the different organisms; also the reactions produced with meat extracts of fresh meat.

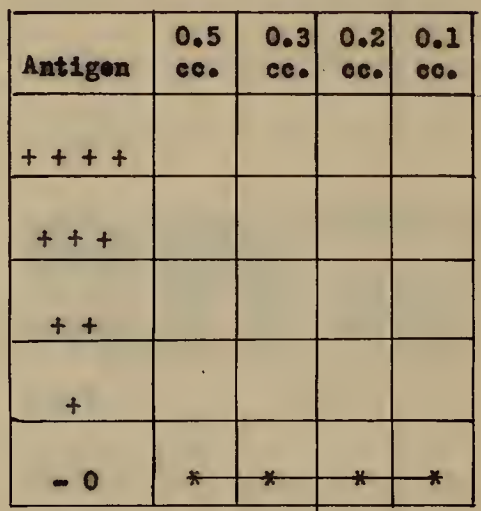
The effect of primary incubation on the sensitiveness of complement-fixation reactions.

The serum of a rabbit immunized against the natural decomposition products of raw beef was employed with a meat extract antigen of meat incubated at 30° C. for 18 hours.

GRAPH I

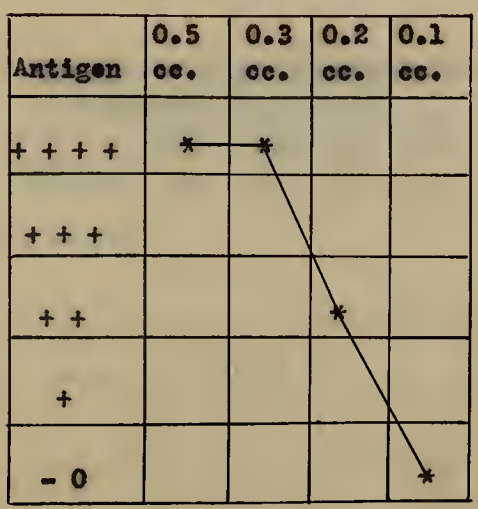
With a primary incubation of one hour at 38° C.

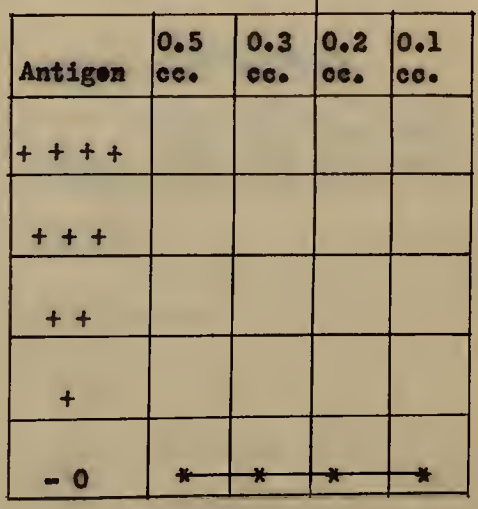




GRAPH II

With a primary incubation of 18 hours at 8° C. - 10° C.



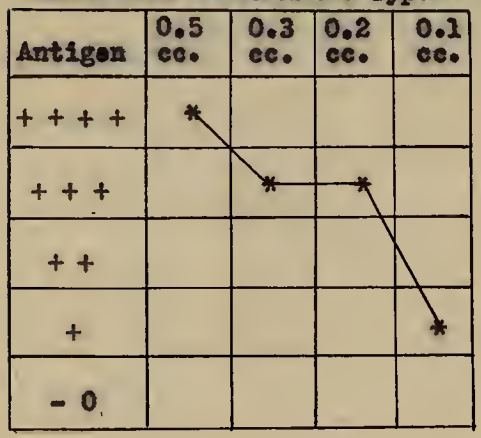


GRAPH III

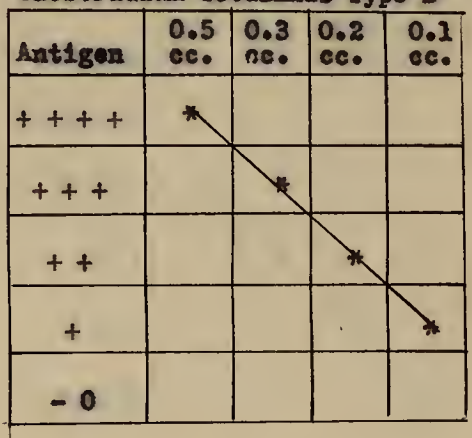
RECORDS OF RESULTS OBTAINED ON COMPLEMENT-FIXATION TESTS WHERE THE SERA OF RABBITS IMMUNIZED AGAINST THE DECOMPOSITION PRODUCTS OF CLOSTRIDIUM BOTULINUS WERE USED WITH VARIOUS MEAT-EXTRACT ANTIGENS.

Serum No. 126-127

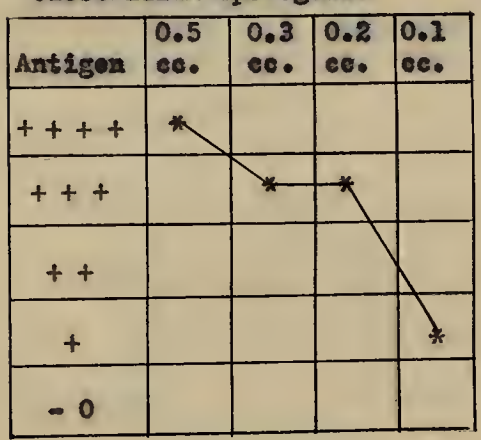
Extract of Meat Inoculated with Clostridium botulinus Type A



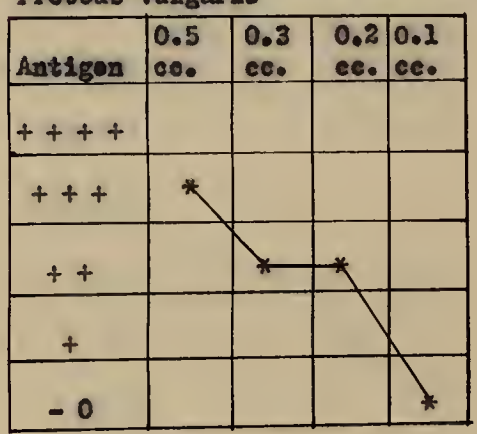
Extract of Meat Inoculated with Clostridium botulinus Type B



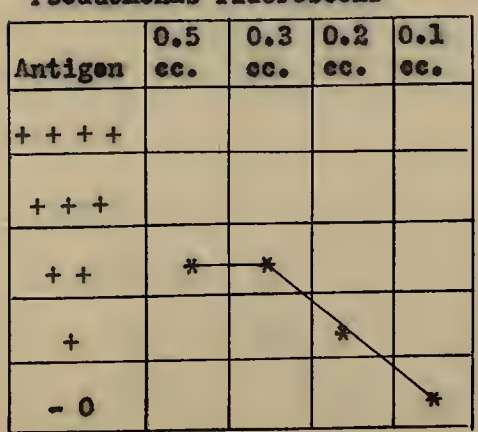
Extract of Meat Inoculated with Clostridium sporogenes



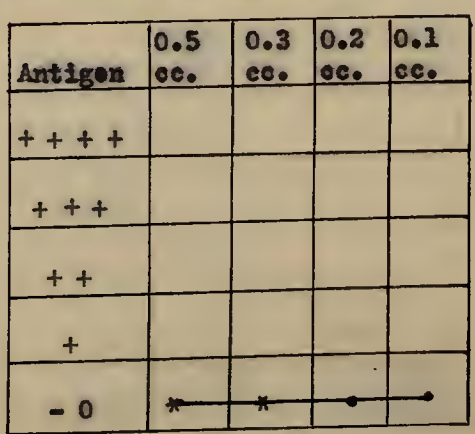
Extract of Meat Inoculated with Proteus vulgaris



Extract of Meat Inoculated with Pseudomonas fluorescens



Extract of Fresh Beef

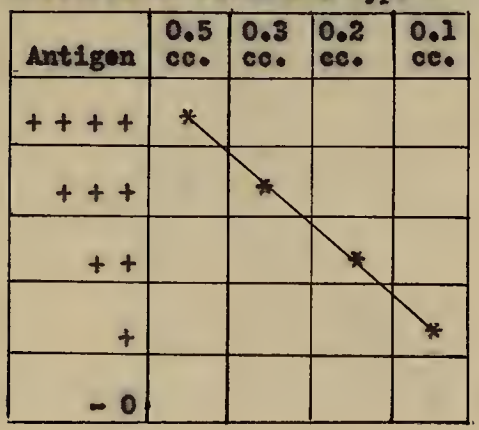


GRAPH IV

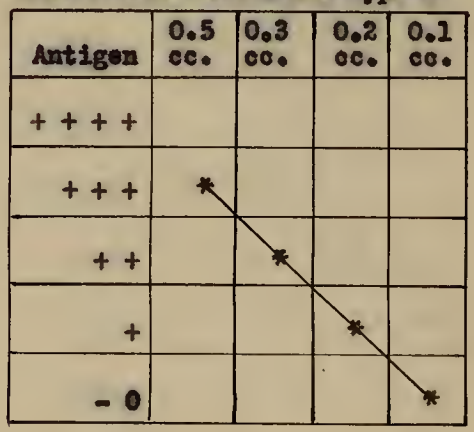
RECORDS OF RESULTS OBTAINED ON COMPLEMENT-FIXATION TESTS WHERE THE SERA OF RABBITS IMMUNIZED AGAINST THE DECOMPOSITION PRODUCTS OF CLOSTRIDIUM BOTULINUS WERE USED WITH VARIOUS MEAT-EXTRACT ANTIGENS.

Serum No. 128-129

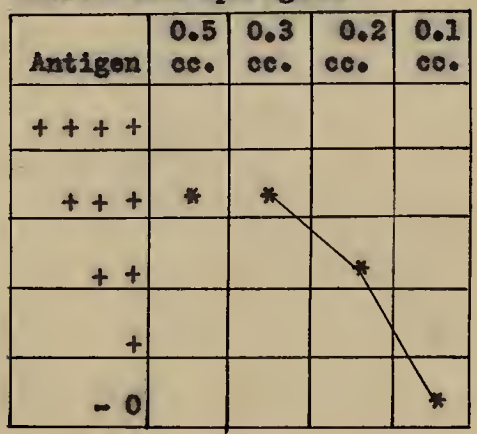
Extract of Meat inoculated with Clostridium botulinus Type A



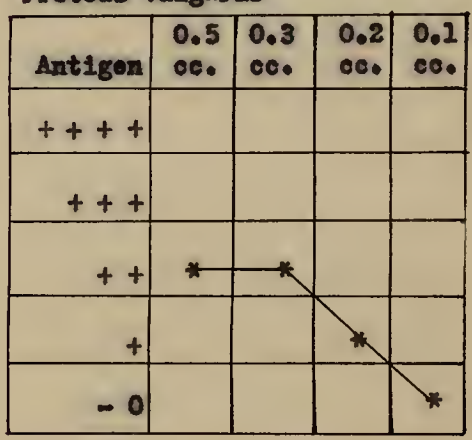
Extract of meat inoculated with Clostridium botulinus Type B



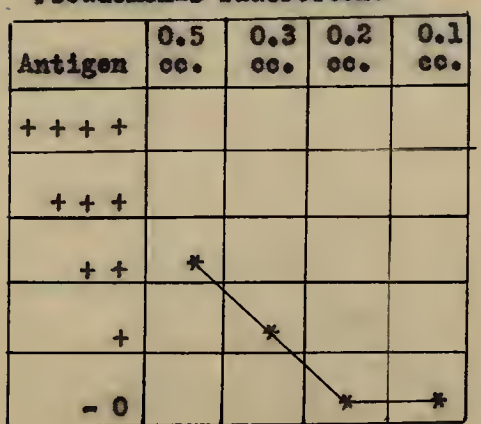
Extract of Meat inoculated with Clostridium sporogenes



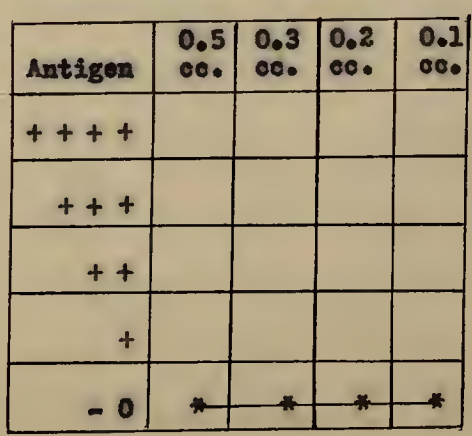
Extract of meat inoculated with Proteus vulgaris



Extract of Meat inoculated with Pseudomonas fluorescens



Extract of Fresh Beef

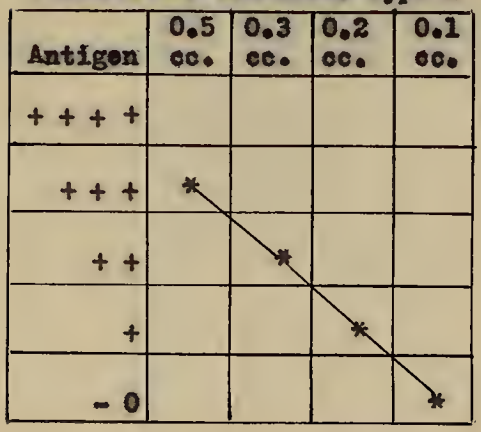


GRAPH V

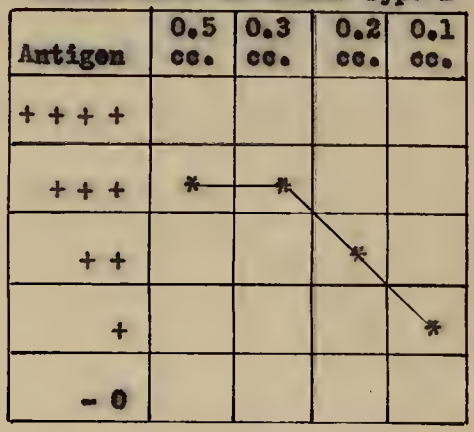
RECORDS OF RESULTS OBTAINED ON COMPLEMENT-FIXATION TESTS WHERE THE SERA OF RABBITS IMMUNIZED AGAINST THE DECOMPOSITION PRODUCTS OF CLOSTRIDIUM BOTULINUS WERE USED WITH VARIOUS MEAT-EXTRACT ANTIGENS.

Serum No. 130-131

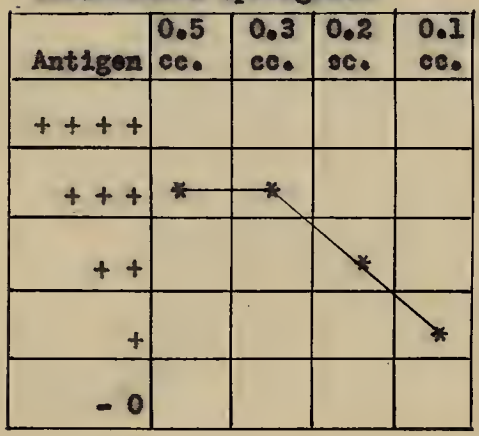
Extract of Meat inoculated with Clostridium botulinus Type A



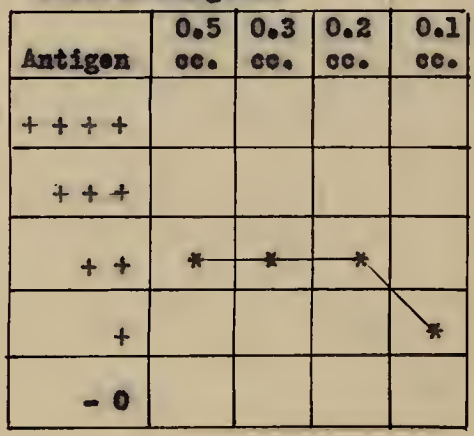
Extract of meat inoculated with Clostridium botulinus Type B



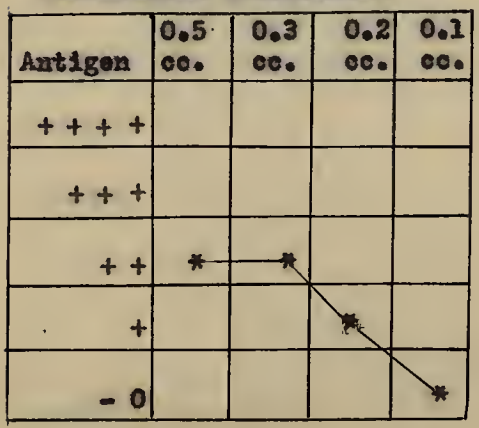
Extract of Meat inoculated with Clostridium sporogenes



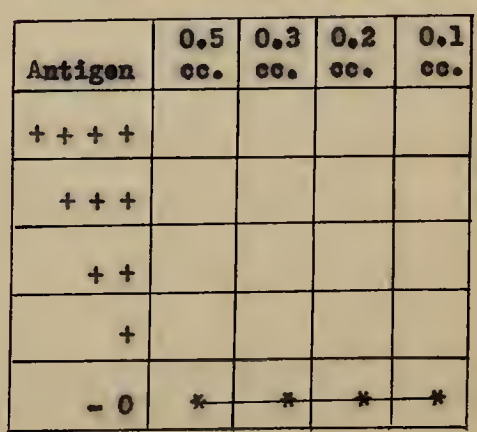
Extract of Meat inoculated with Proteus vulgaris



Extract of Meat inoculated with Pseudomonas fluorescens



Extract of Fresh Beef

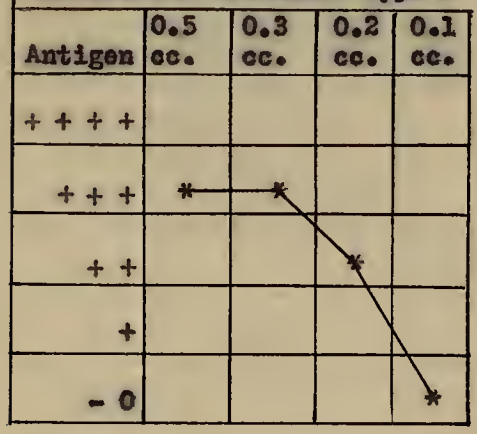


GRAPH VI

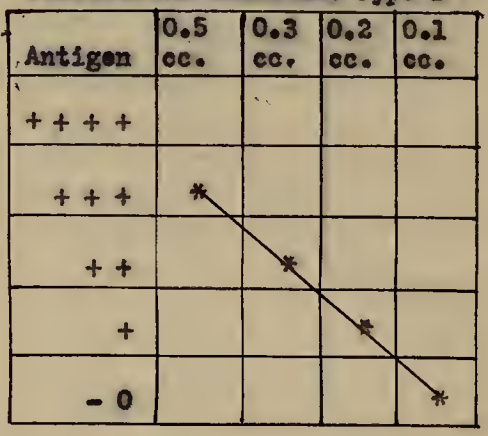
RECORDS OF RESULTS OBTAINED ON COMPLEMENT-FIXATION TESTS WHERE THE SERA OF RABBITS IMMUNIZED AGAINST THE DECOMPOSITION PRODUCTS OF CLOSTRIDIUM BOTULINUS WERE USED WITH VARIOUS MEAT-EXTRACT ANTIGENS.

Serum No. 177-178

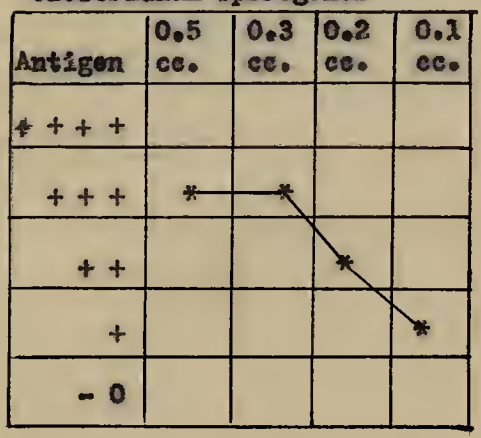
Extract of Meat inoculated with Clostridium botulinus Type A



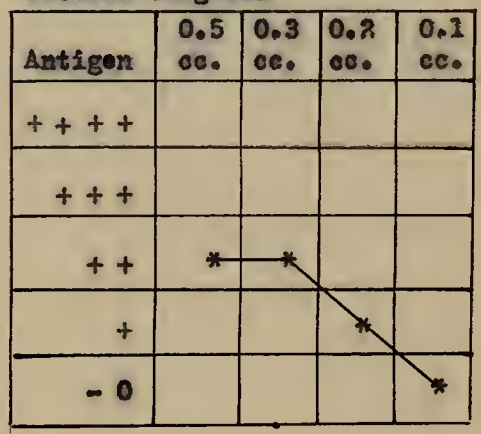
Extract of Meat inoculated with Clostridium botulinus Type B



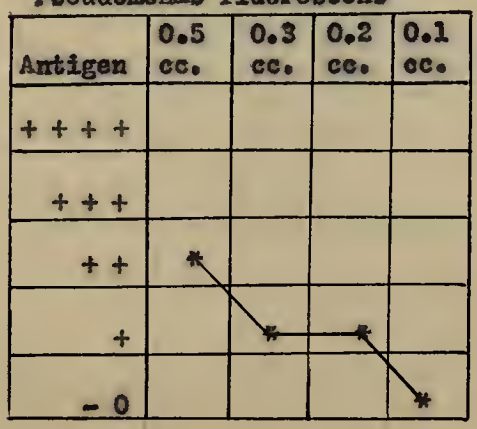
Extract of Meat inoculated with Clostridium sporogenes



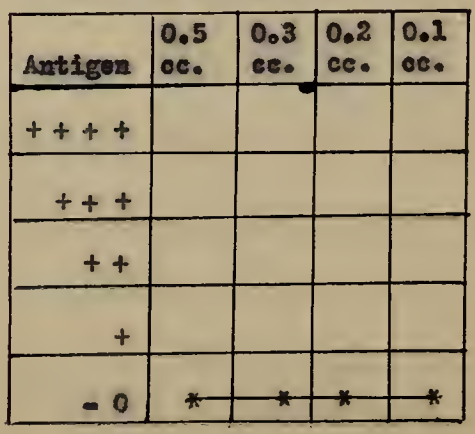
Extract of Meat inoculated with Proteus vulgaris



Extract of Meat inoculated with Pseudomonas fluorescens



Extract of Fresh Beef

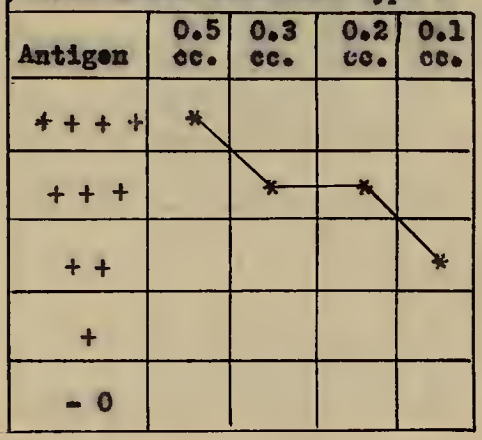


GRAPH VII

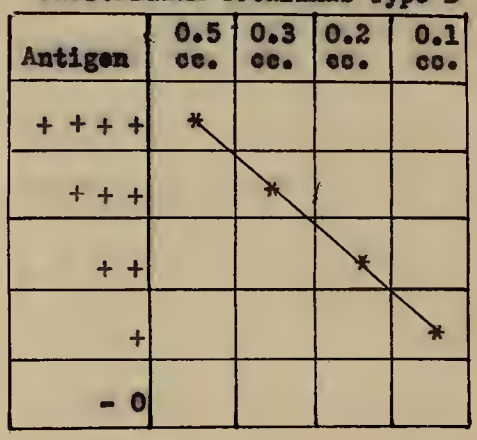
RECORDS OF RESULTS OBTAINED ON COMPLEMENT-FIXATION TESTS WHERE THE SERA OF RABBITS IMMUNIZED AGAINST THE DECOMPOSITION PRODUCTS OF CLOSTRIDIUM BOTULINUS WERE USED WITH VARIOUS MEAT-EXTRACT ANTIGENS.

Serum No. 181-182

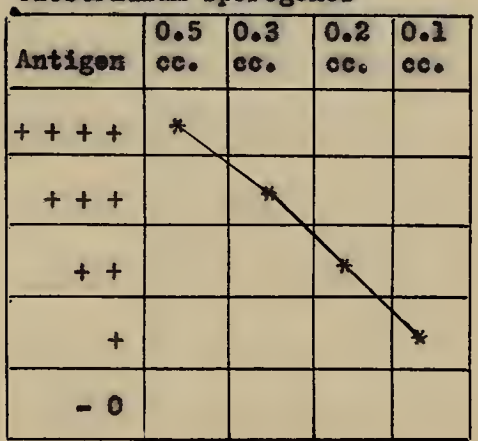
Extract of Meat inoculated with Clostridium botulinus Type A



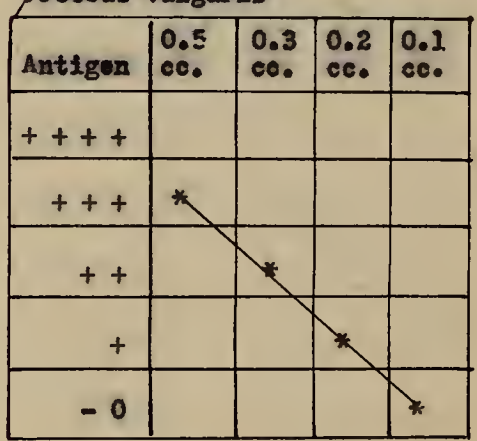
Extract of Meat inoculated with Clostridium botulinus Type B



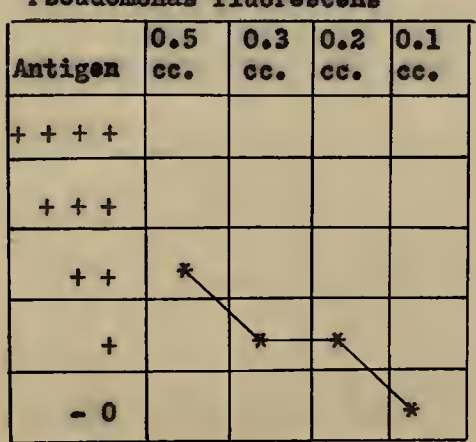
Extract of Meat inoculated with Clostridium sporogenes



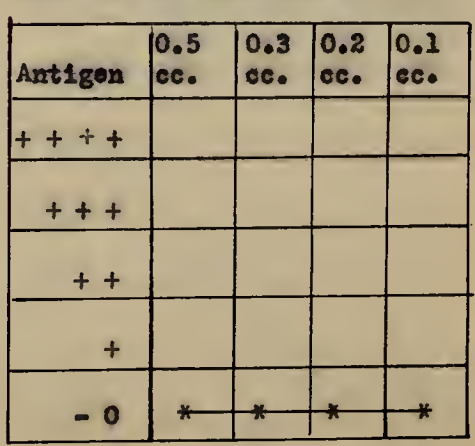
Extract of Meat inoculated with Proteus vulgaris



Extract of Meat inoculated with Pseudomonas fluorescens



Extract of Fresh Beef



GRAPH VIII

On the following graphs are recorded the results of complement-fixation tests when the sera of rabbits immunized against the decomposition products of raw meat were used with antigens of meat showing varying degrees of decomposition.

Serum No. 132-133

Extract of Fresh Meat

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +				
+				
- 0	*	*	*	*

Extract of Meat Allowed to Stand at Room Temperature 24 Hours

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +	*	*		
+ +			*	
+				
- 0				*

Extract of Meat Incubated 24 Hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*	*	
+ + +				*
+ +				
+				
- 0				

Extract of Meat Incubated 16 Hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +	*	*		
+ +			*	
+				*
- 0				

Extract of Fresh Meat

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +				
+				
- 0	*	*	*	*

Extract of Meat Allowed to Stand at Room temperature 16 Hours

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +	*			
+		*		
- 0			*	*

Extract of Meat Inoculated with Proteus vulgaris and incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +				
+ +			*	
+				*
- 0				

Extract of Meat Inoculated with Pseudomonas fluorescens and incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +				
+ +			*	
+				
- 0				*

Extract of Meat Inoculated with Clostridium sporogenes and incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +				
+ +			*	
+				
- 0				*

Extract of Meat Inoculated with Clostridium botulinus and incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +			*	
+ +				
+				*
- 0				

Extract of Meat Incubated for 14 Hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +	*			
+ +		*		
+			*	
- 0				*

Extract of Meat Allowed to Stand at Room Temperature 18 Hours.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +	*	*		
+			*	
- 0				*

GRAPH IX

On the following graphs are recorded the results of complement-fixation tests when the sera of rabbits immunized against the decomposition products of raw meat were used with antigens of meat showing varying degrees of decomposition.

Serum No. 134-135

Extract of Fresh Meat

Antigen	0.5 cc.	0.2 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +				
+				
- 0	*	*	*	*

Extract of Meat Allowed to Stand at Room Temperature 24 Hours

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +	*			
+ +		*		
+			*	
- 0				*

Extract of Meat Incubated 24 Hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*	*	*
+ + +				
+ +				
+				
- 0				

Extract of Meat Incubated 16 Hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*			
+ + +		*		
+ +			*	*
+				
- 0				

Extract of Fresh Meat

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +				
+				
- 0	*	*	*	*

Extract of Meat Allowed to Stand at Room Temperature 16 Hours

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +	*			
+		*	*	
- 0				*

Extract of Meat Inoculated with Proteus vulgaris and Incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*			
+ + +		*		
+ +			*	
+				*
- 0				

Extract of Meat Inoculated with Pseudomonas fluorescens and incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +			*	
+ +				*
+				
- 0				

Extract of Meat Inoculated with Clostridium sporogenes and Incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*	*		
+ + +				
+ +			*	
+				
- 0				*

Extract of Meat Inoculated with Clostridium botulinus and Incubated for 20 hours at 30° C.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +	*			
+ + +		*	*	
+ +				*
+				
- 0				

Extract of Meat Incubated for 14 Hours at 30° C.

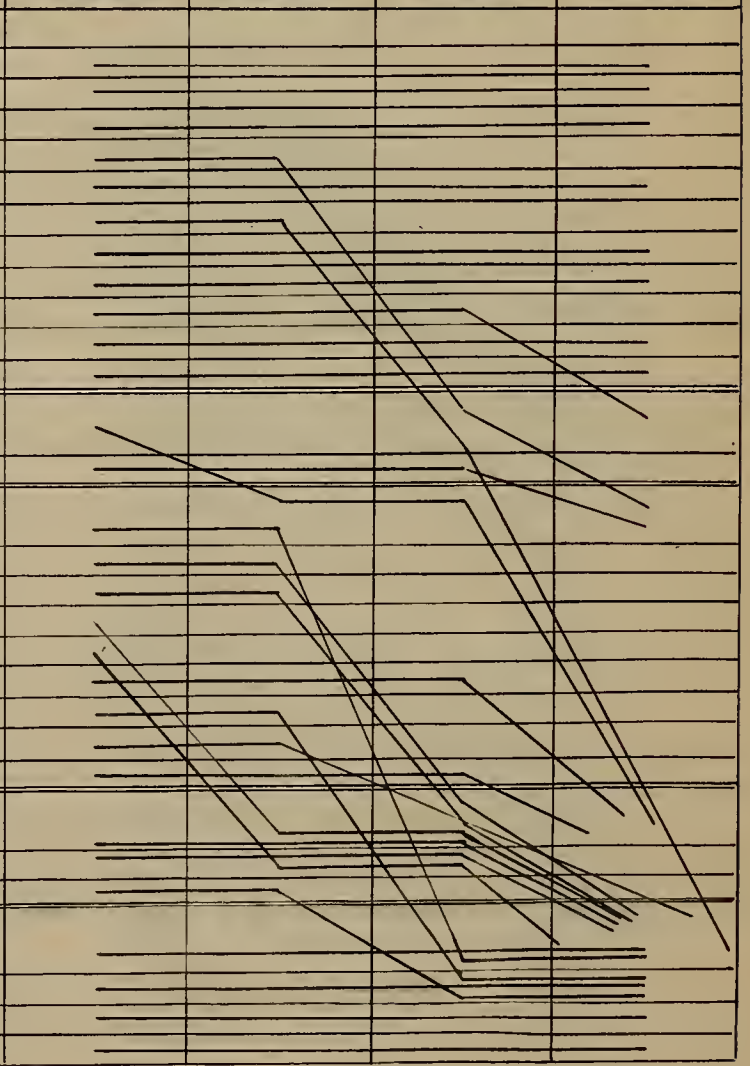
Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +	*			
+ +		*	*	
+				*
- 0				

Extract of Meat Allowed to Stand at Room Temperature 18 Hours.

Antigen	0.5 cc.	0.3 cc.	0.2 cc.	0.1 cc.
+ + + +				
+ + +				
+ +	*			
+		*	*	
- 0				*

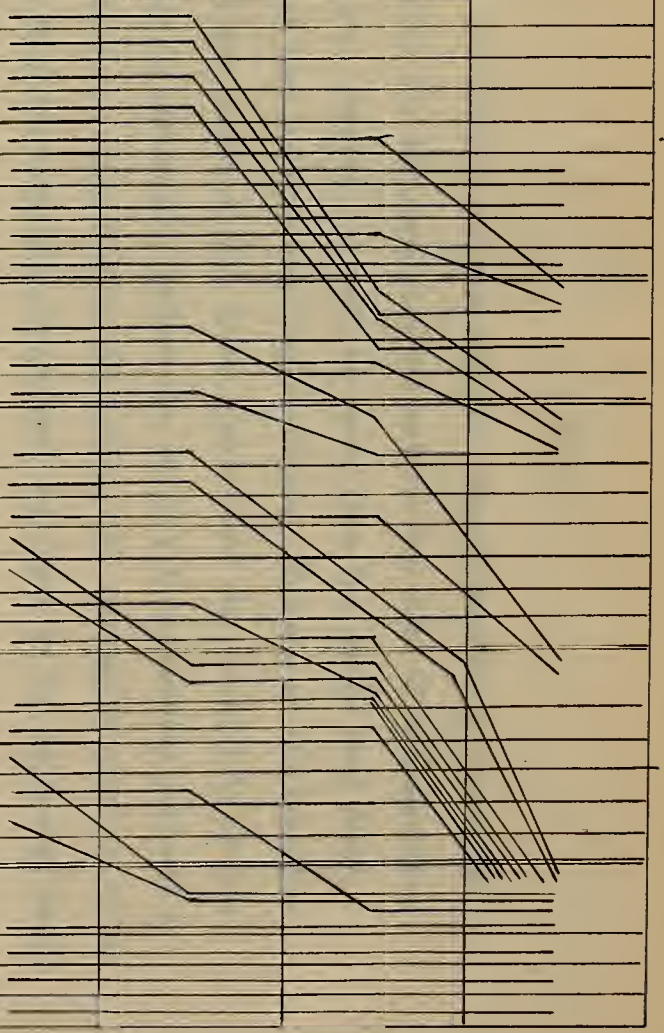
GRAPH X

Meat Extract Antigens Showing Varying Degrees of Decomposition		Tube 1 0.5 cc.	Tube 2 0.3 cc.	Tube 3 0.2 cc.	Tube 4 0.1 cc.
No. 2. Meat incubated for 12 hrs. at 30° C.	+ + + +				
No. 3. Meat incubated for 16 hrs. at 30° C.					
No. 5. Meat incubated for 18 hrs. at 30° C.					
No. 8. Meat incubated for 36 hrs. at room temperature					
No. 10. Meat incubated for 24 hrs. at room temperature					
No. 11. Meat incubated for 16 hrs. at 30° C.					
No. 15. Meat incubated for 12 hrs. at 30° C.					
No. 19. Meat incubated for 36 hrs. at room temperature					
No. 20. Meat incubated for 18 hrs. at 30° C.					
No. 24. Meat incubated for 16 hrs. at 30° C.					
No. 25. Meat incubated for 24 hrs. at room temperature					
No. 30. Meat incubated for 16 hrs. at 30° C.					
No. 7. Meat stored in Frigidaire for two weeks	+ + +				
No. 26. Meat incubated for 22 hrs. at room temperature					
No. 1. Meat incubated for 20 hrs. at room temperature	+ +				
No. 4. Meat incubated for 12 hrs. at 30° C.					
No. 6. Meat incubated for 12 hrs. at 30° C.					
No. 12. Meat incubated for 20 hrs. at room temperature					
No. 13. Meat incubated for 22 hrs. at room temperature					
No. 16. Meat incubated for 10 hrs. at 30° C.					
No. 17. Meat incubated for 20 hrs. at room temperature					
No. 22. Meat incubated for 22 hrs. at room temperature					
No. 28. Meat incubated for 10 hrs. at 30° C.					
No. 9. Meat incubated 18 hrs. at room temperature	+				
No. 21. Meat incubated 10 hrs. at 30° C.					
No. 29. Meat incubated 18 hrs. at room temperature					
No. 14. Fresh Meat	- 0				
No. 18. Fresh Meat					
No. 23. Fresh Meat					
No. 27. Fresh Meat					



GRAPH XI

Meat Extract Antigens Showing Varying Degrees of Decomposition		Tube 1 0.5 cc.	Tube 2 0.3 cc.	Tube 3 0.2 cc.	Tube 4 0.1 cc.
No. 3. Meat incubated for 12 hrs. at 30° C.	+ + + +				
No. 5. Meat incubated for 16 hrs. at 30° C.					
No. 8. Meat incubated for 18 hrs. at 30° C.					
No. 10. Meat incubated for 36 hrs. at room temperature					
No. 11. Meat incubated for 24 hrs. at room temperature					
No. 19. Meat incubated for 16 hrs. at 30° C.					
No. 20. Meat incubated for 12 hrs. at 30° C.					
No. 24. Meat incubated for 36 hrs. at room temperature					
No. 25. Meat incubated for 18 hrs. at 30° C.					
No. 30. Meat incubated for 16 hrs. at 30° C.					
No. 2. Meat incubated for 24 hrs. at room temperature	+ + +				
No. 15. Meat incubated for 16 hrs. at 30° C.					
No. 26. Meat stored in Frigidaire for two weeks					
No. 1. Meat incubated for 22 hrs. at room temperature	+ +				
No. 4. Meat incubated for 20 hrs. at room temperature					
No. 7. Meat incubated for 12 hrs. at 30° C.					
No. 13. Meat incubated for 12 hrs. at 30° C.					
No. 21. Meat incubated for 20 hrs. at room temperature					
No. 22. Meat incubated for 22 hrs. at room temperature					
No. 28. Meat incubated for 10 hrs. at 30° C.					
No. 6. Meat incubated for 20 hrs. at room temperature	+				
No. 9. Meat incubated for 22 hrs. at room temperature					
No. 12. Meat incubated for 10 hrs. at 30° C.					
No. 16. Meat incubated for 18 hrs. at room temperature					
No. 17. Meat incubated 10 hrs. at 30° C.					
No. 29. Meat incubated 18 hrs. at room temperature					
No. 14. Fresh Meat	- 0				
No. 18. Fresh Meat					
No. 23. Fresh Meat					
No. 27. Fresh Meat					



The results obtained with the above procedure proved to be specific for decomposition but not for the type of decomposition present. However, it will be noted from the graphs that fresh cooked beef yielded negative reactions. Because of the negative reactions produced with fresh cooked beef the possibility of producing an ambocoptor against the natural decomposition products of raw beef was suggested to the author, the amboceptor produced to be used in differentiating fresh beef from that in the beginning stages of decomposition.

Preparation of the Antigen Used in the Immunization of Rabbits to Produce Antibodies Against Natural Decomposition Products of Beef:

For this purpose raw lean freshly ground hamburger steak was selected. Twenty-gram portions of the beef were placed in large sterile test tubes; these were stoppered with cotton plugs and incubated at 30° C. for 72 hours in a saturated atmosphere. The decomposed beef was then extracted with 120 cc. of saline and centrifuged for one-half hour at high speed. The supernatant liquid was then filtered through a Seitz filter and the clear filtrate used as the antigen for immunization.

Immunization of the Rabbits:

Two large healthy rabbits weighing 2200 and 2600 grams were given four intravenous inoculations as described before in amounts of 2, 2, 3, and 6 cc. of the antigen, and five subcutaneous inoculations in amounts of 8, 10, 12, 15 and 15 cc. The last five inoculations were given subcutaneously because of the large quantity of antigen used.

The animals were allowed to rest for a week and were then bled from the marginal vein of the ear and the blood tested for antibody content as described before. As specific antibodies for the antigen used in immunization were found to be present, the rabbits were bled to death and the serum preserved with phenol and ampouled in 2 cc. quantities.

Preparation of Antigen to be Tested:

Antigens prepared from decomposed raw beef were found to be more anticomplementary than those from the cooked beef so it was found necessary to use a method in preparing the antigens which would reduce their anticomplementary properties.

Ten grams of the meat to be tested were thoroughly extracted with 50 cc. of saline. The extract was centrifuged for 10 minutes to remove any solid particles; fat which rose to the top was removed and the supernatant liquid was placed in a test tube and heated in a water bath at 56° C. for 15 minutes. The extract was then filtered through medium grade filter paper and the clear filtrate used as the antigen.

TABLE VII

The Effect of Heat on Anticomplementary Properties of Antigens

Antigen - An Extract of Meat decomposed for 24 hours.			
Antigen Unheated		Antigen Heated at 56° C. for 15 min.	
0.5 cc. Antigen	Blocking of Complement Produced by the Antigen	0.5 cc. Antigen	Blocking of Complement Produced by the Antigen
1:2 dilution	Inhibition of hemolysis	1:2 dilution	Slight inhibition of hemolysis (unit)
1:4 "	Slight inhibition of hemolysis	1:4 "	Complete hemolysis
1:6 "	Slight inhibition of hemolysis (unit)	1:6 "	Complete hemolysis
1:8 "	Complete hemolysis	1:8 "	Complete hemolysis
1:10 "	Complete hemolysis	1:10 "	Complete hemolysis
1:12 "	Complete hemolysis	1:12 "	Complete hemolysis
1:16 "	Complete hemolysis	1:16 "	Complete hemolysis
1:20 "	Complete hemolysis	1:20 "	Complete hemolysis
1:32 "	Complete hemolysis	1:32 "	Complete hemolysis
1:40 "	Complete hemolysis	1:40 "	Complete hemolysis
Antigen - An Extract of Meat Decomposed for 36 hours.			
0.5 cc. Antigen	Blocking of Complement Produced by the Antigen	0.5 cc. Antigen	Blocking of Complement Produced by the Antigen
1:2 dilution	Inhibition of hemolysis	1:2 dilution	Inhibition of hemolysis
1:4 "	Inhibition of hemolysis	1:4 "	Slight inhibition of hemolysis (unit)
1:6 "	Slight inhibition of hemolysis	1:6 "	Complete hemolysis
1:8 "	Slight inhibition of hemolysis (unit)	1:8 "	Complete hemolysis
1:10 "	Complete hemolysis	1:10 "	Complete hemolysis
1:12 "	Complete hemolysis	1:12 "	Complete hemolysis
1:16 "	Complete hemolysis	1:16 "	Complete hemolysis
1:20 "	Complete hemolysis	1:20 "	Complete hemolysis
1:32 "	Complete hemolysis	1:32 "	Complete hemolysis
1:40 "	Complete hemolysis	1:40 "	Complete hemolysis

From Table VII it will be seen that heating the antigens to 56° C. for 15 minutes reduced their anticomplementary properties, thus increasing their antigenic index or the ratio between the antigenic unit and the anticomplementary unit.

The Quantitative Complement-fixation Test for Detecting the Presence of Occult Decomposition in Raw Beef:

With the following established modifications the author proposes the following method as an established procedure for detecting the presence of decomposition in raw beef.

0.5 cc. of immune serum of a 1 to 10 dilution (amount carrying one unit), inactivated at 62° C. for one-half hour was placed in each of four test tubes for each of the antigens to be tested.

The object of inactivating the serum at 62° C. for one-half hour was to eliminate any chances of non-specific reactions which normal sera would produce. From Table VI the reader may observe a record of results obtained when using normal rabbit serum inactivated at 55° C. for 15 minutes, with antigens of fresh and decomposed beef, also results obtained when normal rabbit serum inactivated at 62° C. for 20 minutes was used with antigens of fresh and decomposed beef. It will be noted that non-specific reactions were entirely eliminated by heating the serum at 62° C. for 30 minutes.

A dilution of the antigen to be tested, prepared and heated at 56° C. for 15 minutes as previously described, in which 0.5 cc. contained one-half of its anticomplementary unit was prepared, and 0.5 cc., 0.3 cc., 0.2 cc., and 0.1 cc. quantities were added to each of four test tubes.

Sufficient saline was added to each to make the total volume to one cc. From Table VII it will be noted that heating the antigens at 56° C. for 15 minutes greatly diminished their anticomplementary properties; the remainder of this property was removed subsequently by dilution.

The tubes were allowed to stand 5 to 10 minutes, then one cc. of diluted complement carrying two full units was added to each. These were mixed and incubated in a water bath for one hour at 38° C. or in the Frigidaire at 10 C. for 16 to 18 hours so as to ensure a fixation of complement if the reaction was positive.

After the primary incubation the hemolytic system was added; if incubated in the Frigidaire the tubes were warmed in the water bath for 10 minutes, before adding the hemolytic system.

The contents of the tubes were mixed and reincubated in a water bath for one hour at 38° C. Three controls were set up, one on the serum, one on the antigen, and one on the hemolytic system.

Complement-fixation reactions for detecting the presence of decomposition in beef carried out by the above procedure exhibited specificity in differentiating between fresh beef and that which had decomposition products present, even though present in small quantities. This may be observed from the accompanying graphs.

DISCUSSION

Spoilage of meat is a complex process; there may be a souring of meat due to the fermentation of carbohydrates caused by the aerobes, anaerobes and facultative anaerobes, a digestion of proteins caused by these microorganisms resulting in the production of cleavage products of protein and in end products of putrefaction and also a digestion of the protein by autolysis of the meat.

These products of decomposition as revealed by the experimental results have the power of stimulating antibody formation in animals. The antibodies formed were of such nature that they could be detected by complement-fixation. The nature of these antigenic substances was not determined. However, it is known that carbohydrates and the products of carbohydrate metabolism are in themselves non-antigenic, also that the end products of protein digestion or the amino acids are non-antigenic; therefore these factors could not be the stimulating substances in antibody formation. Obermayer and Pick[24] have found that of the protein molecule it is the aromatic radicle which is the determining factor in the specificity of immunological reactions. Vaughn[38] believes that of the toxic and non-toxic fractions of protein cleavage, the toxic fraction contains the aromatic radicle and it is this fraction which is the determining factor in stimulating antibody formation. True toxins are known to stimulate the formation of antitoxins, but a toxin-antitoxin reaction does not involve a third component or complement so a reaction of this order would not be demonstrable by complement-fixation. In this particular project the reaction would not be of this order as a fixation of complement takes place. The antigenic substance responsible for the stimulation of antibody formation must, therefore, be cleavage products of proteins in

such combinations that they have the characteristics essential for antigenicity. They may be amino acids containing aromatic radicles in combination with amino acids of the aliphatic series, the combination having large molecular dimensions and exhibiting colloidal properties.

In the present investigation, the antibody formation was low, probably due to a low concentration of these antigenic substances in the meat extract antigen used as the inoculum. If these substances could have been determined and used in a concentrated form in immunizing the animals, it is quite possible that a serum with a high antibody content could have been produced and perhaps the addition of some accessory protein substance such as peptone, which would act as an activator, would also have increased the antibody formation.

The same problem of low antigenic substances had to be met when testing meat extracts for evidence of decomposition. These substances present in the meat extracts are variable and present in amounts according to the degree of decomposition. For this reason it was necessary to increase the antigenic index of the antigen, either by increasing the antigenic sensitiveness or decreasing the anticomplementary properties; the latter was done as it was found that heating the antigens to 56° C. for 15 minutes decreased their anticomplementary properties.

Reference to Table VII will illustrate the effect of heat in decreasing the anticomplementary properties of meat extract antigen.

Lack of specificity as to type of decomposition present in the meat extract antigens was probably due to the similarity in the metabolism of the proteolytic organisms.

Wagner and Meyer[39] have shown, through a comparative analysis of the metabolic products of Clostridium botulinus, Clostridium sporogenes and Clostridium histolyticus, in meat and milk media, that the biochemical activities of these organisms are similar.

Complement-fixing antibodies are ordinarily highly resistant to deleterious influences. Their resistance to heat is probably one of their most important properties because of the custom of heating sera to 55° C. preliminary to testing. Natural complement-fixing antibodies are usually more susceptible to heat than those produced by artificial immunization or during disease. The effect of varying degrees of heat on antibodies appears to depend on the nature of the antibody and on the time of exposure.

In working with syphilis antibodies, Sachs[40] has found that if syphilitic sera is heated at 62° C. for 30 minutes the complement-fixing antibody is destroyed. Marie and Levaditi[41] report that the syphilis antibody in cerebrospinal fluid was destroyed by heating the fluid at 75 to 80° C. for 20 minutes.

Kolmer, Rule and Trist[42] Have shown that destruction of syphilis antibody may begin when the sera is heated at 40° C. for 30 minutes and that the destruction progresses with higher temperatures. When sera is exposed for 30 minutes at 62° C. these investigators have found that approximately 35 per cent of the sera showed a total loss of syphilis antibodies.

In rabbit immune sera, however, complement-fixing antibodies show a much greater resistance to heat. Kolmer[7] in using rabbit sera

containing anti-human, anti-dog, anti-cat and other antibodies for the detection and differentiation of the various proteins found that such sera heated at 62° C. for 30 minutes have rarely shown any deterioration of antibody content, this being a fortunate circumstance as it has been found necessary to heat rabbit sera at 62° C. for 30 minutes in order to eliminate non-specific reactions.

In working with the antibodies against the decomposition products of beef the author has obtained results which confirm with those of Kolmer. The antibodies produced by immunizing rabbits against the decomposition products of beef have been found to be stable when exposed to a temperature of 62° C. for 30 minutes. This procedure being necessary in order to eliminate non-specific reactions as the reader may observe from Table VI.

The method of primary incubation employed is an important factor in determining the sensitiveness of a reaction.

In working with the Wassermann reaction the majority of investigators have observed stronger reactions with a primary incubation at a low temperature for several hours than when incubated at 38° C. for one hour. The possibility of the existence of two kinds of antibodies, one fixing complement best at 38° C. and the other at a lower temperature, has been advanced. However, Kolmer, Matsunami, Toitsu and Trist[43] conducted complement-fixation tests for syphilis using a primary incubation at 38° C. for one hour and a second set at 8° to 10° C. for 18 hours using the same sera, antigens and technique and obtained no evidence indicating the existence of two kinds of antibodies. The only differences encountered were that with some sera, very weak in antibody content, positive reactions were obtained when tested at 8° C. to 10° C. for 18 hours and negative reactions when tested at 38° C. for one hour.

In working with meat extract antigens the author was also able to obtain more sensitive reactions by using a primary incubation of 18 hours at 8 to 10° C., than by a water bath incubation at 38° C. for one hour.

However, with the meat extract antigens the prolonged incubation is not justifiable for the slight increase in sensitiveness.

The results recorded on Graphs I and II show the effect of primary incubation on the sensitiveness of the reaction produced.

From Graphs I to XI inclusive it will be noted that with fresh meat extracts used as antigens all reactions obtained were negative, while the degree of positiveness exhibited with extracts of decomposed beef varied with degree of decomposition. The variance in the degree of fixation is due to variability of the antigenic substances present in the extracts. The specificity of these reactions was demonstrated by duplicate tests performed by two different individuals, using the same antigens and the same technique. The antigens consisted of 30 meat extracts exhibiting varying degrees of decomposition, also extracts of fresh beef.

A comparison of Graphs X and XI will show that the efficiency of the test was found high, as in both tests no extracts from decomposed beef failed to show antigenic properties specific for the immune serum, while the reactions with fresh beef were all negative.

The results of this investigation appear to warrant the use of complement-fixation in determining decomposition in beef as this method directly indicates decomposition, while bacterial counts and other methods advanced are only indirect indicators of putrefaction, with the

exception of the organoleptic and the ammonia test which are too liberal as the meat is actually putrid before it responds to them.

The author is confident that with further studies on the antigen used for immunization in the project, results may be derived which will produce a diagnostic serum with a high enough antibody content that the procedure as given will be practical as a laboratory test.

SUMMARY

From the studies and results obtained in this investigation the following summary appears justified:

1. The products of decomposition of both cooked and raw beef have the power of stimulating antibody formation in susceptible animals.

2. The antibodies formed are of such nature that they may be detected by complement-fixation reactions.

3. The immune serum produced by the immunization of rabbits is specific for decomposition of beef, giving a fixation of complement when used with meat extracts having these products of decomposition present.

4. This same immune serum yields negative reactions when used with an extract of fresh beef or one in which the protein has not been hydrolyzed or decomposed.

5. Since the immune serum yields positive reactions with meat extract antigens containing decomposition products and negative reactions with meat extracts of fresh beef, the antibodies present must be against the cleavage products of the proteins of the beef and not against the whole proteins of the meat.

6. The reaction as carried out proved to be non-specific as to the organism producing the decomposition; aerobic and anaerobic decomposition could not be differentiated.

7. With the modifications on the procedure as outlined, the results obtained indicate that complement-fixation may be used as a means of detecting minute quantities of decomposition in beef.

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