Recent Developments Concerning the Anaerobic Bacteria and their Activities, with Particular Reference to the Tetanus and Gangrene Organisms*

L. S. McClung, Indiana University

Although anaerobic bacteria were recognized much earlier and some studies of their importance had been made, it was not until the time of the first world war that sufficient impetus was given to these investigations to bring forth proper methods of study and a clear picture of their relationship to disease and certain processes in the economy of nature. By 1918 there were available techniques for the isolation and study of an anaerobe though some of the techniques now appear clumsy and open to question. The tetanus organism had been isolated and correctly associated with disease and some of the gangrene organisms had been recognized. The antitoxin for the tetanus organism was available for prophylactic or therapeutic purposes but the toxin of the Welch bacillus had been obtained only recently and the possibility of the preparation of an antitoxin investigated. The Weizmann process for the production of butyl alcohol and acetone by anaerobic fermentation of starchy materials was an outgrowth of the need during the war for acetone for the manufacture of explosives. In 1919 Burke, on the basis of toxin neutralization (by antitoxin) studies, divided the botulinus group into two types.

Many advances have been made concerning these organisms since 1914-18 and it may be of interest to summarize certain of the important relationships. Since limitations of time will not permit the historical development of any of the topics, only the most recent research will be discussed and for the same reason only certain topics will be chosen for discussion. Since the literature on the anaerobic organisms accumulates roughly at the rate of seven hundred and fifty to one thousand papers per year, obviously only a fraction of these may be discussed. Those interested in additional material are referred to the subject bibliography of the literature on these organisms (78, 80). A recent monograph (155) is devoted to the anaerobic bacteria but unfortunately it lacks conciseness and may also be criticised for the inclusion of much material reported in the literature which has not been accepted by other investigators.

Methods and Media for the Study of Anaerobic Bacteria

There have been several recent advances in the techniques for the study of anaerobic bacteria, and through these the technician in the laboratory is now able to work with the anaerobes almost as quickly and easily as with the aerobic types. These and older methods are given in detail in the new edition of Leaflet III of the Manual of Methods of

* Address of the retiring chairman of the Bacteriology Section.

Pure Culture Study (75), but it may be well to discuss briefly some of the newer techniques. One of the oldest systems for securing an anaerobic environment made use of the absorption of oxygen in the reaction between pyrogallic acid and alkali. Spray (139), using this reaction, designed a deep bottomed petri dish which has a ridge across the center to keep solutions of the two chemicals separated until the top and bottom of the dish are sealed together by tape or plasticene. Thus each dish is a separate unit. The Pyrex dish, called the Bray dish, is similar to the original Spray dish and is to be preferred. Another single plate system, which was described only recently by Brewer (14), has much to recommend it. This consists of a special top of heavy glass and the design of the dish is such that the top of the dish rests, at its periphery, on the agar medium (in the bottom dish) to form a seal, and the remainder of the dish is slightly raised. Thus only a small amount of air is trapped over the surface of the agar and this is removed by means of the reducing action of the medium. For satisfactory use of these dishes a medium of high reducing capacity is necessary such as that, supplied by the Baltimore Biological Laboratories, in which sodium thioglycollate and sodium formaldehyde sulfoxylate are incorporated. Vera (149) has found the plate quite useful in the demonstration of hemolysis by anaerobic bacteria.

A recent reintroduction has been made by Marshall and Nordby (85) of the use of an aerobic organism to utilize the oxygen and provide anaerobic conditions for single plate cultures. The system proposed uses small plates for the aerobic organism which are pressed down into the agar of the regular plate and this latter plate is inoculated with the anaerobic species. The aerobic organism uses the oxygen in the small area enclosed between the surface of the two agar plates.

For the incubation of a number of tubes or plates of the ordinary type there is now available a jar made in this country called the Brewer jar (17) which is similar in principle to the McIntosh and Fildes jar which was originally available only in England. An advantage of the Brewer jar is that ordinary illuminating gas may be used in place of hydrogen for flushing the jar prior to combustion. It is not difficult to manipulate and its purchase is suggested to those who have a large number of anaerobic plates in routine use.

Rosenthal (122) has proposed another method for securing anaerobic conditions based upon the liberation of hydrogen by the action of sulphuric acid on powdered chromium. This is an easy system for the production of hydrogen and the ingredients for the reaction are usually available. Miles (89) objected to the method on the basis of the probable evolution of a gas, possibly hydrogen sulphide, which was toxic to certain important organisms. Mueller and Miller (93) modified the original method by including sodium carbonate to liberate carbon dioxide and their system is sufficiently simple to merit mention. Either a desiccator equipped with a stopcock to provide for escape of the gases or a two quart fruit jar with a metal casting of the lid to which is attached an escape spout may be used. For the fruit jar one gram of sodium carbonate and three grams of powdered chromium (98% pure) are placed

in the bottom of the jar after which the cultures are added, and then 30 ml. of a solution (15% by volume) of sulphuric acid are introduced by means of a funnel. The lid is clamped on immediately. Directions are included in the original paper for a simple mercury trap which allows escape of the gases and yet prevents inflow of oxygen.

With reference to special media for anaerobic organism in recent years we note a growing use of liver infusion (77, and others) for the enrichment of many species particularly for the types producing butyric acid and butyl alcohol and for one of the thermophilic species which is involved in the spoilage of canned foods. It is probable that the success of the liver infusion for these organisms relates to the need for certain growth factors which are supplied by the liver tissue. A very inexpensive medium, simple to prepare, for use for general purpose was suggested (77) which combines the growth promoting qualities of the liver tissue with the semisolid nature of 5% corn meal mash. The finished medium has the virtue of remaining anaerobic for extended periods even without special seals at the surface to protect the medium from absorption of oxygen.

One of the most notable of advances in late years in the problem of the easy cultivation of anaerobic species is the proposal by Brewer (12, 13) of the use of sodium thioglycollate in liquid media to serve as the major reducing agent in the "aerobic" cultivation of anaerobes. Other compounds including thioglycollic acid had been proposed for the same purpose but most of these were thermolabile and hence it was necessary to add these to the media aseptically following sterilization. The sodium thioglycollate is, however, heat stable and may be incorporated with the other ingredients at the time of preparation of the medium. Brewer also proposed a nutrient base medium containing the reducing agent and a small percentage of agar, and claimed that the medium could be used successfully without a special anaerobic seal. With the exception of certain specialized meat infusion media and a few others the practice of "open" incubation of anaerobic media had not been practiced generally for most media become reoxidized almost immediately after sterilization. The claims of Brewer have been fully substantiated. Marshall, Gunnison and Luxen (84) found the medium suitable for sterility testing of biological products. The National Institute of Health recognized the use of the medium for this purpose (35). McClung (73, 76) showed that the new medium compared favorably with meat infusion and other media for the enrichment of *Clostridium welchii, C. septicum, C. tetani* and other pathogenic species when only a small number of cells were present in the inoculum. Further it was found (74) that the use of sodium thioglycollate aided in the problem of a suitable medium for organisms with a long lag period and in the preparation of large volumes of certain anaerobic cultures. Others (7, 30, and 102) have discussed the problem of the neutralization of mercurials by the medium. Mollov, Winter and Steinberg (90) claimed distinct advantages for the use of thioglycollate media in routine blood and post-mortem cultures involving the isolation of strains of pneumococci and streptococci. Brewer (15) has proposed an adidtional compound, sodium formaldehyde sulfoxylate, is

a reducing agent but to date no other studies concerning this compound have appeared.

Spray (140) has published an extensive paper on the use of semisolid tubed media in the study of anaerobic species in which he has shown that the addition of a small percentage of agar is sufficient to prevent the immediate reoxygenation of the media following the reduction in oxygen potential which occurs during heat sterilization. With this aid he has proposed formulae for media for physiological reactions which may be inoculated and incubated without a necessity of placing the tubes in an anaerobic jar or using a seal of vaseline or other material at the surface of the liquid in each tube. The convenience of this method when it is compared with some of the older methods is not easily underestimated. Unfortunately the scheme of species identification which was proposed by Spray involves a two week incubation for completion of certain of the differential reactions. Reed and Orr (118) have combined the semisolid agar technique of Spray (140) with the thioglycollate reducing agent of Brewer (12, 13) to good advantage and have proposed formulae of new media and a system of rapid identification of the gas gangrene anaerobes. Their paper is recommended for study by all who deal with this problem in the clinical laboratory.

Accessory Growth Factors and Synthetic Media Problems

One or more accessory growth factors were early shown to be required by *C. sporogenes* (65), *C. botulinum* (36), members of the butyric-butyl group (111, 161), non-sporulating types (157, 158), and possibly other species. Of special interest in this problem are the recent reports concerning *C. tetani* and *C. welchii*. A study of the possibilities of growth and toxin production on synthetic media were, as we shall see later, incident to investigations on the proper media for the production of toxoids.

As an extension of his studies on the nutrition of the diphtheria bacillus, Mueller and his associates initiated a series of very fruitful investigations. In 1940 (91) it was reported that tetanus toxin could be produced on a simplified medium. Mueller and Miller (92, 94, and 95) later obtained growth on a medium composed of the usual inorganic elements, an acid hydrolysate of protein, tryptophane, adenine, or hypoxanthine, pantothenic acid, thiamine, riboflavin, "folic" acid. Biotin was listed as probably being essential. Another contribution (96) gives the formula for the production of tetanus toxin in a peptone free broth. With mice the potency was in the range of m.l.d. = approximately 160,000. For this the nitrogen source was a dilute casein hydrolysate and to this were added appropriate amounts of the following: (1) Accessories and metals mixture containing magnesium sulphate, traces of copper, manganese, and zinc, and nicotinic acid, beta alanine and pimelic acid, (2) cystine, (3) tryptophane, (4) glucose, (5) calcium pantothenate, and (6) liver eluate. As with the diphtheria bacillus the concentration of iron was shown to be a critical factor and directions for removing the excess iron were included.

The problem of the production of *C. welchii* toxin on a simplified medium has been attacked by several investigators. Basu and Sen (3), using a veal infusion broth, were able to produce filtrates with the average m.l.d. stated to be 0.00005 ml. for a 350 gram guinea pig. Taylor and Stewart (147) proposed a medium of Bacto-peptone, casein, potassium bicarbonate, ammonium dihydrogen phosphate, disodium hydrogen phosphate, ferric ammonium citrate, and glucose. Seal (127) used a digest of veal and fresh beef liver.

Tamura, *et al.* (145, 146) have given information on the production of *Clostridium welchii* toxin in a peptone free medium. An acid hydrolysate of casein was used with addition of various salts, tryptophane, panthothenic acid, riboflavin, nicotinic and pimelic acids and biotin. Again it was shown that minute concentrations of iron influenced the production of toxin.

Species Identification Reactions

Of major importance and interest are reactions or a small group of procedures which enable the laboratory technician to make a rapid identification of a new isolation. These are perhaps of special importance in the identification of members of the gas gangrene group. What procedures are available for use with important species of the genus *Clostridium*? We have mentioned earlier the general identification outlines proposed by Spray (140) and Reed and Orr (118). An earlier procedure, proposed by Hall (54), gave considerable emphasis to the morphology of the spore. In addition to these general tests, and others such as characteristic pathology observed in artificially infected animals, there have been proposed, though not as yet universally accepted, certain specific procedures which merit brief discussion.

The "stormy milk" fermentation for the identification of *C. welchii* doubtless is familiar to all. It has been noted by several workers that this reaction alone must not constitute a complete diagnosis due to the fact that certain other species, principally motile butyric acid and butyl alcohol producing species also may give a "stormy fermentation" of milk particularly if the inoculum contains a considerable number of cells. These are differentiated by positive motility and by inability to produce toxin. Robinson and Stovall (121) proposed that the reaction could be made more specific if 1.0 ml. of 20% Na_2SO_3 and 0.1 ml. of 8% $FeCl_3$ were added to each 10 ml. of milk and noted that in the modified medium *C. welchii* gave a blackening reaction.

It may be remembered also that the English bacteriologists Wilson and Blair in the early 1920's proposed a bismuth sulphite medium for the recognition of *C. welchii* in contaminated water supplies. Lyons and Owen (71) have examined the possible use of this medium in the clinic. According to them the Wilson-Blair medium is a useful diagnostic aid in the early recognition of the presence of certain clostridia in wound exudates but has the disadvantage that there is no correlation between toxigenicity and the production of the characteristic reaction.

Another specific reaction, generally termed the Nagler reaction, has been proposed for *C. welchii* which has aroused considerable interest.

In 1939, Seiffert (129), in Germany, and Nagler (99) from Australia, independently reported that a mixture of *C. welchii* toxin and normal human serum produced upon incubation of a characteristic opalescence. Mcfarlane, Oakley and Anderson (81) believed that this opalescence was due to the liberation of insoluble fatty material from the serum and showed also that a dilute solution of egg yolk, called lecithovitellin, could be substituted for the human serum. Nagler (100) agreed that this substitution was satisfactory and also used the reaction as an indicator in toxin-antitoxin titrations. Oakley and Warrock (103), Seal and Stewart (128) and Stewart (142) are others who have used the reaction. Hayward (57) modified the reaction by the use of a fluid Nagler test medium into which isolated colonies from the original sample plating could be picked. Thus it was claimed that certain identification of *C. welchii* could be completed within 40-48 hours after plating of the original sample. Hayward reported also preliminary details of the use of the reaction with solid media and extended these observations in the Medical Research Council's War Memorandum No. 2 (87). In her latest paper (59), there is a critical examination of the specificity of the plate reaction and a recommendation of the plate method for the routine examination of wound and puerperal swabs from patients suspected of having anaerobic infections. Certain streptococci and a few aerobic and anaerobic spore-bearers also give zones of opacity but the formation of these is not inhibited by antitoxin. The *Sordelli-bifermentans* group give zones which are neutralizable by *welchii* antitoxin but these reactions are usually feeble. Further, these organisms may be differentiated from *C. welchii* by the presence of spores on 24 hour plate cultures and by failure of lactose fermentation.

When considering the Nagler reaction it should be noted that Weed and his associates (151, 152, 153) have questioned the specificity of both the reaction of *welchii* toxin with lecithovitellin and of its neutralization by *welchii* antitoxin. They contribute evidence to show that the flocculation develops as a result of acid production during growth and that many species, both aerobic and anaerobic, would cause a reaction. Hayward (59) believes that the results obtained by Weed and associates are due in the main to peculiarities of the broth media which they used. Although the final word on the suitability of the Nagler reaction as a specific diagnostic test for *C. welchii* must be delayed until Weed has had an opportunity to examine his data in the light of the suggestions of the English workers, it seems possible that out of the controversy there may arise a test which will be of value in the clinic.

A different type of a cultural reaction was proposed by Gordon and McLeod (50) as a simple and rapid method for distinguishing *C. novyi* (*C. oedematiens*) which is another of the gas gangrene anaerobes. On a medium containing benzidine and a peroxidase they noted a blackening reaction which they recommended as a useful method of identification of *C. novyi* in pathologic material. It was claimed that the only other organisms which were found to produce the blackened area were *C. botulinum* and some strains of anaerobic streptococci. Hayward (58), however, did not confirm these data but found several organisms were

capable of producing the blackening and some of these were difficult to differentiate from *C. novyi.* Further, all colonies of *C. novyi,* even from a virulent strain, did not always produce the characteristic blackening. These observations of Hayward are in agreement with some observations in our laboratories (113), so we must conclude that, at the present at least, the reaction proposed by Gordon and McLeod should be used with caution.

The last suggestion concerning recognition of the toxin producing species of *Clostridium* has appeared only recently from the English group. Petrie and Steabben (112), using as a base medium a glucose horse-meat infusion broth made with Evans peptone, added to this medium, immediately prior to pouring, an amount of the appropriate antitoxin to give a final concentration of eight international units per ml. A zone of precipitation occurs around the colony. Using antitoxin for the different species in separate plates early recognition of the various organisms was claimed.

Serological Studies with Anaerobes

In comparison with the knowledge of the 1918 era we have come a long way on the road in the late years. To date, however, I cannot claim that either the precipitin reaction or the complement fixation have contributed much useful information with the anaerobic species, but there is quite a different story with regard to the agglutination and the toxin-antitoxin reactions. We have reviewed (79) the entire story but it may be well to outline some of the advances here and to consider more recent material.

With regard to the agglutination reaction, in my opinion, the possible usefulness of this test has increased considerably following the recognition that it was possible to differentiate the somatic and flagellar antigens of motile anaerobes in a manner similar to the well known studies on the enteric organisms. No claims have yet been made that these reactions are useful in the diagnosis of anaerobic infection in which the serum of the patient would be used but the considerable data of importance have accumulated with reference to the study of pure cultures of certain species.

The results with *C. tetani* are especially interesting. It was early shown that subgroups of the species existed on the basis of the flagellar antigen and Gunnison (52) in a study of 67 strains found evidence for nine groups (and a tenth has been added later). Further, the results indicated the presence of a common O antigen which showed no type specificity. Certain of the groups established on the flagellar antigen reactions showed an additional O antigen. Related species did not possess the O factor for *C. tetani* and it was shown that certain atoxic strains could be recognized by their reaction in serum prepared from a known strain.

Subdivision of *C. septicum* on the basis of agglutination reaction also relates to the flagella antigen. At least four, possibly six, groups are apparent (4, 31). Cross reactions with the O antigens of these groups

reveal close relationship but not identity of the factors. The somatic antigen fraction of *C. chauvoei* likewise appears distinct and offers possibilities of a method of separation of this organism.

C. parabotulinum is another species in which we find (72) subdivisions based upon the flagellar antigens whereas there seems to be a somatic antigen common to all strains. Some cross reaction is obtained with *C. sporogenes*, but a *parabotulinum* serum pre-absorbed by *sporogenes* offers distinct possibilities as a reagent for species diagnosis.

What of the agglutination of *C. welchii*—one of the most important species of the genus? There were early claims that it was impossible to produce an agglutination antiserum for this organism. It should be remembered that this species is non-motile and should show reactions only in the somatic series. Late experiments show that this is true and several investigators (62, 154, 160) have been able to obtain satisfactory titers. It seems, however, that there are a number of subgroups (104) and to date no useful aid has been suggested as a result of the new data.

All of the available evidence indicates that the species *C. tetani, C. septicum, C. histolyticum* are monotypic with respect to toxin formation. That is to say, the antitoxin from the toxin of any strain will neutralize the toxin of any other strain. This simple story is, however, not the case with *C. welchii.* On the basis of non-cross neutralization tests it is now recognized that there are four groups within the organisms having physiological reactions similar or identical with the organism known as *C. welchii.* These were proposed first as distinct species being called—*Bacillus agni, Bacillus paludis* and *Bacillus ovitoxicus.* Wilsdon (160), however, studying representatives of each of these has concluded that it would be better to consider these as representing four toxin types within the same species, in as much as the differences in physiology between these organisms were considered to be of minor importance. These are now generally known as Type A, B, C, and D of *C. welchii,* or *C. perfringens* if you choose to follow the latest edition of Bergey.

It is important to note that thus far only toxin Type A has been associated with gas gangrene in man whereas the other types are the agents of various animal diseases. Type B is concerned with an enterotoxemia of young sheep and has been called the lamb dysentery bacillus. Type C was recovered from another sheep disease called *struck* which seems localized in England. The last organism Type D has its origin in animal disease also being associated with enterotoxemia of sheep in West Australia and a disease aptly termed "pulpy kidney".

A great deal has been written on the fractionation on basis of physiological reaction of the toxins of these four types and it seems likely that the entire picture is not yet completely clear. The majority of the early literature was reviewed in 1938 by McCoy and McClung.

But one of the gangrene organisms yet remains for discussion. This one was not described until 1922 and has had a rather checkered history since then. Originally described by Sordelli, it was given a trinomial name, *Clostridium oedematis sporogenes* which name, being invalid, was changed to *Bacillus sordelli.* Other workers isolated in this country a gangrene organism from a post-operative infection (and also the catgut

used) to which they gave the name *Clostridium oedematoides*. There now seems to be no question but that these organisms are identical but lately there seems reason to suspect that this pathogenic species may be closely related to, if not identical with, the non-pathogenic species *Clostridium bifermentans*. This question is now being considered in our laboratories with a collection of authentic strains collected from the original authors or other official sources.

To turn back to the 1918 era for a brief moment it will be recalled that Burke in 1919 subdivided the organisms producing the botulinus toxin into two groups or types—designated A and B. We now find that this type differentiation has been extended to five (A through E) and that a new advance is the recognition of the fact that two widely divergent physiological types are concerned (See 79 for literature citations). Some of the strains are proteolytic while others are non-proteolytic. The majority of the strains of American origin are in the former group, but it should be noted that recent cases of the disease in America were thought (40, 60) to have arisen from Type E toxin—a type known previously only from Russia. This may account for failure of therapeutic use of antitoxin in some cases since the majority of this is produced against only Type A and Type B toxin. The Type E organism is non-proteolytic, somewhat difficult to grow, and not so greatly heat resistant. A very real problem is presented to the laboratory in the isolation of this organism from sample material. Thus far the best procedure concerns itself with the identification of the toxin from the original sample by means of animal protection tests.

So much for the laboratory study of the organisms and their reactions. Let us now consider briefly a few other points. Perhaps the greatest advance since the time of the last war with reference to the anaerobic infections and intoxications relates to the successful use of injection of toxoid as a means of stimulation of active immunity against tetanus. At the present time there is available both plain toxoid and alum precipitated toxoid for this purpose. We note that the French, Italian, American and probably the German armies have instituted tetanus toxoid injections for all and the British have it available though the vaccination, as is usual, is not compulsory. At least in the American Forces the Navy and Marine Corps are similarly protected. In this country there is divided opinion among the forces as to the type of toxoid to be used (31, 101, 132, 133)—the Army using plain or liquid toxoid whereas the Navy and Marine Corps specify alum precipitated toxoid. Regardless of this divergent opinion there is ample evidence that toxoid injections are effective in stimulating a basic immunity which should be effective in preventing the disease (25, 32, 45, 63, 86, 109, 110, and others).

Usually the routine consists of two injections of toxoid two to three months apart and then a third "booster" or "recall response" dose is given after a considerable period (144) or in the Armed Forces just prior to the entrance of the person, who is receiving the injections, into an active combat zone. Gold (46, 47, 48) has advocated the stimulation of active immunity by the combined subcutaneous and intranasal routes. Tetanus toxoid may be combined with typhoid vaccine (37, 51, 83, 115,

116), with diphtheria toxoid (5, 24, 38, 117) and the suggestion has also been made (108, 143) that gangrene and tetanus toxoid may be combined.

In addition to its use in the Armed Forces it may be noted that toxoid immunization has been recommended for children and for allergic individuals (38, 39, 67, 109, 110, 148) and others who may be exposed to probable sources of infection. It would seem that a sound basis exists for its use rather than the possible sensitization of large groups as a result of prophylactic injections of antitoxin. It must be remembered that tetanus is not confined to injuries following battle wounds but that it does occur also in civilian life.

Of importance is the question that has been raised concerning the production of toxoid from a toxin produced on a medium containing peptone (particularly Witte peptone) due to the sensitization which may result (2, 16, 26, 49, 105, 114, 123, 159). This has been satisfactorily answered for other media are now available which do not contain peptone, and it is claimed (16) that the use of alum precipitated toxoid avoids sensitization. In particular we would mention again the work of Mueller and associates who have shown that toxin produced on their peptone free medium can be converted to toxoid (126) and further that such toxoid is antigenic (97, 98). Fraser, *et al.*, (37) also show that toxin prepared on peptone free media can be used without the production of anaphylactic reactions. It has been recommended, however, that skin tests for sensitivity be employed routinely and/or that adrenaline or epinephrine be kept at hand whenever injections are made.

A means of solving the question of avoiding serum reactions following the use of the usual equine antitoxin in prophylactic injections has been considered also. Glaser (41, 42) has proposed the use of bovine antitoxin for this purpose while Schaeffer and Myers (125) successfully treated a patient with a despeciated antiserum. The antitoxin in the latter case had been subjected to partial digestion by takadiastase.

The possibilities of the production of an effective toxoid with *C. welchii* toxin has been investigated. Laboratory studies with animals are in the affirmative and it may be expected that these results have been extended to man but to date extensive studies on this have not appeared (66, 106, 107, 142).

What of other means of protection against these anaerobic infections or of treating them? One thinks, of course, of the sulfonamide drugs as chemoprophylactic and chemotherapeutic agents. Sulfonamide drugs are now supplied in sterile form to each U. S. soldier who is instructed to dust the material in wounds, his own or of others, if medical aid is not immediately available following injury. Local treatment seems more effective than oral but the results are complicated due to the fact that the various species of anaerobes differ in their sensitivity to these drugs (6, 8, 19, 21, 23, 53, 56, 68, 70, 82, 119, 134, 135, 141) and to zinc peroxide which has been proposed also (88, 120). There is some evidence perhaps contrary to what you would expect, that these drugs are contraindicated when serum therapy is instituted.

Sterile drugs were mentioned above. Peculiarly there exists the possibility that the drug itself might be contaminated at times and would

thus introduce rather than prevent an infection (1, 156). Adequate means, however, are now available for heat sterilization of the sulfonamides (18, 22, 69).

X-ray has been proposed as a beneficial diagnostic and therapeutic aid for gas gangrene and several reports have appeared concerning this topic (9, 10, 11, 20, 27, 33, 43, 44, 55, 124, 130, 131, 136). Not all the evidence is in favor of the therapeutic value (20, 28, 137, 150) though Kelly (64), the main proponent of its use presents a convincing summary of his and other work. The increase of gas in the tissue in early stages of the infection following an injury as revealed by successive x-ray pictures would seem a valuable aid in diagnosis. In this regard it must be remembered that the diagnosis of gangrene must often be based largely on clinical evidence, not bacteriological, due to the time required for the results of bacteriological tests and also since it has been shown that *C. welchii* may exist in a wound which shows no evidence of gangrene (61, 138).

References

1. Atkinson, E. C., 1942. Tetanus after sulphonamide treatment of cavities. Brit. Med. J., **2**:24-25.
2. Auld, A. G., 1940. Anaphylaxis after injection of tetanus toxoid. Brit. Med., J., **1**:368.
3. Basu, P. N., and Sen, S. N., 1941. A peptic digest broth for the formation of *Clostridium tetani* toxin. Indian J. Med. Research, **29**:689-690.
4. Bengtson, I. A., 1933. The potency testing of *Vibrion septique* antitoxin. J. Bact., **25**:84-85.
5. Bigler, J. A., and Werner, M., 1941. Active immunization against tetanus and diphtheria in infants and children. J. Am. Med. Assoc., **116**:2355-2366.
6. Bliss, E. A., Long, P. H., and Smith, D. G., 1941. Chemotherapy of experimental gas gangrene and tetanus infections in mice. War Med., **1**:799-810.
7. Blubaugh, L. V., and Reed, W., 1943. Sodium thioglycollate as an antibacteriostatic agent. Its use in sterility testing. J. Bact., **45**:44.
8. Bonnin. N. J., and Fenner, F., 1941. Local implantation of sulphanilamide for the prevention and treatment of gas gangrene in heavily contaminated wounds: A suggested treatment for war wounds. Med. J. Aust., **1**:134-140.
9. Bowen, A., 1940. Roentgen therapy in gas gangrene. Experience at Station Hospital, Eighth Corps Area, Fort Sam Houston. Mil. Surg., **86**:107-112.
10. Brailsford, J. F.. 1940a. Gas gangrene. Brit. Med. J., **1**:503.
11. Brailsford, J. F.. 1940b. X rays in diagnosis and treatment of gas gangrene. Brit. Med. J., **1**:247-249.
12. Brewer, J. H., 1940a. A clear liquid medium for the "aerobic" cultivation of anaerobes. J. Bact., **39**:10.
13. Brewer, J. H., 1940b. Clear liquid mediums for the "aerobic" cultivation of anaerobes. J. Am. Med. Assoc., **115**:598-600.
14. Brewer, J. H., 1942a. A new petri dish cover and technique for use in the cultivation of anaerobes and microaerophiles. Science, **95**:587.
15. Brewer, J. H., 1942b. Further studies on media containing reducing agents. J. Bact., **43**:36.
16. Brown, H. H., 1940. Anaphylaxis after injection of tetanus toxoid. Brit. Med. Jour., **2**:683.
17. Brown, J. H., and Brewer, J. H., 1938. A method for utilizing illuminating gas in the Brown, Fildes, and McIntosh or other anaerobe jars of the Laidlaw principle. J. Lab. and Clin. Med., **23**:870-874.
18. Buckland, W. J., 1942. Sterilizing sulphanomide powders. Brit. Med J., **2**:264.
19. Buttle, G. A. H., 1940. Chemotherapy of infected wounds. Lancet (London), **1**:890-893.

20. Caldwell, G. A., 1942. The prevention and treatment of gas gangrene. Experimental observations on the use of sulfonamides, roentgen ray, and zinc peroxide. Tri-State Med, J., **15**:2803-3806.
21. Caldwell, G. A., and Cox, F. J., 1942. Gas gangrene: Experimental observations on the use of the sulfonamide derivatives and zinc peroxide in its treatment and prevention. Discussion. South. Med. J., **35**:789-798, 951.
22. Cherry, J. C., 1942. Tetanus after sulphonamide treatment of cavities. Brit. Med. J., **2**:142.
23. Committee on Chemotherapeutic and Other Agents and the Subcommittee on Infectious Diseases, of the Division of Medical Sciences, National Research Council, 1941. Chemotherapy for infectious diseases and other infections. War Med., **1**:55-65.
24. Cooke, J. V., 1938. Combined active immunization for diphtheria and tetanus. A plea for its routine use. South. Med. J., **31**:158-161.
25. Cooke, J. V., and Jones, F. G., 1943. The duration of passive tetanus and its effect on active immunization with tetanus toxoid. J. Amer. Med. Assoc., **121**:1201-1209.
26. Cooke, R. A., Hampton, S., Sherman, W. B., and Stull, A., 1940. Allergy induced by immunization with tetanus toxoid. J. Amer. Med. Assoc., **114**: 1854-1858.
27. Cooper, R. W., 1941. The use of x-ray therapy in the treatment of gas bacillus infection. Tri-State Med. J., **13**:2769-2771.
28. Cozen, L., 1940. The therapy of gas gangrene. Mil. Surg., **87**:231-232.
29. Cunningham, A. A., 1940. Anaphylaxis after injection of tetanus toxoid. Brit. Med. J., **2**:522-523.
30. Daily, F. K., and Blubaugh, L. V., 1941. The elimination of bacteriostatic action by the use of sodium thioglycollate medium. J. Bact., **42**:147-148.
31. Davesne, J., 1928. Races sérologiques du *Vibrion septique*. Compt. Rend. Soc. Biol. (Paris), **99**:763-764.
32. Deamer, W. C., Bates, G., and Smyth. F. S., 1942. The initial response to immunization with diphtheria and tetanus alum toxoid. J. Pediatrics, **20**:169-181.
33. Dowdy, A. H., and Sewell, R. L., 1941. Roentgen radiation in experimental *Clostridium welchii* infection (gas gangrene) in dogs: Preliminary report. Radiology, **37**:440-442.
34. Enders, J. F., 1942. The present program for the immunization of military personnel. New England Jour. Med., **227**:162-165.
35. Federal Register, 1942. **7**:No. 26. 781-782.
36. Fildes, P., 1935. The tryptophan and "sporogenes vitamin" requirements of *B. botulinus*. Brit. J. Expt. Path., **16**:309-314.
37. Fraser, D. T., MacLean, D. L., Orr, M. D., Plummer, H. C., and Wishart, F. O., 1943. Tetanus toxoid and its use for active immunization. Am. J. Pub. Health, **33**:1107-1114.
38. Friedman, T. B., Bigler, J. A., and Werner, M. A., 1942. The immunologic response of allergic children to toxoid. J. Allergy, **13**:114-124.
39. Gallagher, J. P., Gallagher, C. D., and Haufmann, G. G., 1942. Tetanus toxoid injection of adolescents. New England J. Med., **227**:691-694.
40. Geiger, J. C., 1941. An outbreak of botulism. J. Amer. Med. Assoc., **117**:22.
41. Glaser, J., 1941. The use of bovine antitoxin for the prophylaxis of tetanus. J. Pediatrics, **19**:403-413.
42. Glaser, J., 1940-41. The use of bovine antitoxin for the prophylaxis of tetanus. Discussion. J. Allergy, **12**:537-542.
43. Godby, W. H., 1940a. Treatment of gas gangrene. Brit. Med. J., **1**:107.
44. Godby, W. H., 1940b. X-rays in the treatment of gas gangrene. Med. J. Aust., **1**:85-88.
45. Gold, H., 1937. Studies on tetanus toxoid. II. Active immunization of normal persons with tetanus toxoid, alum precipitated, refined. Discussion. J. Am. Med. Assoc., **109**:481-484.
46. Gold, H., 1938. Active immunization of allergic individuals against tetanus by means of tetanus toxoid, alum precipitated refined. J. Allergy, **9**:545-550.

47. Gold, H., 1940. Active immunization against tetanus by the combined subcutaneous and intranasal routes. A simple procedure for the maintenance of a protective antitoxin titer. Amer. J. Surg. n. s., **48**:359-375.
48. Gold, H., 1940-41. Active immunization against tetanus by the combined subcutaneous and intranasal routes. Discussion. Penn. Med. Jour., **44**:1565-1569.
49. Gold, H., 1941-42. Sensitization induced by tetanus toxoid, alum precipitated. J. Lab. and Clin. Med., **27**:26-36.
50. Gordon, J., and McLeod, J. W., 1940. A simple and rapid method of distinguishing *Cl. novyi (B. oedematiens)* from other bacteria associated with gas gangrene. J. Path. and Bact., **50**:167-168.
51. Grasset, E., and Girdwood, A. I., 1940. Active tetanus immunization and combined tetanus-typhoid immunization with tetanus anatoxin and typhoid endotoxoid vaccine. So. African Med. J., **14**:169-175.
52. Gunnison, J. B., 1937. Agglutination reactions of the heat stable antigens of *Clostridium tetani.* J. Immunol., **32**:63-74.
53. Hac, L. R., Eilert, M. L., Adair, F. L., 1942. Chemotherapy in *Cl. welchii* infection in mice. Soc. Expt. Biol. and Med., Proc., **51**:108-110.
54. Hall, I. C., 1922. Differentiation and identification of the sporulating anaerobes. J. Infect. Diseases, **30**:445-504.
55. Ham, H., 1940. Gas gangrene infection treated by deep x-ray therapy. Med. J. Aust., **2**:287-288.
56. Hawking, F., 1941. Prevention of gas-gangrene infections in experimental wounds by local application of sulphonamide compounds and by sera. Brit. Med. J., **1**:263-268.
57. Hayward, N. J., 1941. Rapid identification of *Cl. welchii* by the Nagler reaction. Brit. Med. J., **1**:811-814, 916.
58. Hayward, N. J., 1942. The use of benzidine media for the identification of *Clostridium oedematiens.* J. Path. and Bact., **54**:379-382.
59. Hayward, N. J., 1943. The rapid identification of *Cl. welchii* by Nagler tests in plate cultures. J. Path. and Bact., **55**:285-293.
60. Hazen, E. L., 1942. Differential characters of two strains of *Clostridium botulinum.* Soc. Expt. Biol. and Med. Proc., **50**:112-114.
61. Hill A. M., and Butler, H. M., 1942. Bacteriological investigations in *Clostridium welchii* infection. Med. J. Aust., **1**:55.
62. Howard, A., 1928. Races sérologiques du *B. perfringens.* Ann. Inst. Pasteur, **42**:1403-1419.
63. Jordan, E. P., and Halperin, G., 1941. Tetanus toxoid for prophylaxis. War Med., **1**:227-246.
64. Kelly, J. F., and Dowell, D. A., 1941. Twelve-year review of x-ray therapy of gas gangrene. Discussion. Radiology, **37**:421-439.
65. Knight, B. C. J. G., and Fildes, P., 1933. A vitamin necessary for the growth of *B. sporogenes:* Its relation to auxin and other growth factors. Brit. J. Expt. Path., **14**:112-124.
66. Kolmer, J. A., 1942. Toxoids in active immunization against gas gangrene. J. Immunol., **43**:289-296.
67. Lapin, J. H., 1942. Combined immunization of infants against diphtheria, tetanus and whooping cough. Am. J. Diseases Children, **63**:225-237.
68. Long, P. H., 1940. The clinical use of sulfanilamide and its derivatives in the treatment and prophylaxis of certain infections. N. Y. Acad. Med. Bul., **16**:732-750.
69. Long, P. H., 1942. Sterilization of sulphonamides. Lancet (London) **2**:322-323.
70. Love, R. J. M., 1940. Treatment of gas gangrene with sulphapyridine. Brit. Med. J., **1**:908.
71. Lyons, C., and Owen, C. R., 1942. Wilson-Blair medium in the rapid diagnosis of the clostridia of gas gangrene. J. Bact., **43**:685-687.
72. McClung, L. S., 1937. Studies on anaerobic bacteria. X. Heat stable and heat labile antigens in the botulinus and related groups of spore-bearing anaerobes. J. Infect. Diseases, **60**:122-128.

73. McClung, L. S., 1940a. The use of dehydrated thioglycollate medium in the enrichment of spore-forming anaerobic bacteria. J. Bact., **40**:645-648.
74. McClung, L. S., 1940b. The use of sodium thioglycollate in culturing large volumes of anaerobic bacteria. Science, **92**:340.
75. McClung, L. S., Committeeman on Anaerobic Methods, 1943a. The study of obligately anaerobic bacteria. Leaflet III, 4th edition, Pure Culture. Study of Bacteria.
76. McClung, L. S., 1943b. Thioglycollate media for the cultivation of pathogenic clostridia. J. Bact., **45**:58.
77. McClung, L. S., and McCoy, E., 1934. Studies on anaerobic bacteria. I. A corn-liver medium for the detection and dilution counts of various anaerobes. J. Bact., **28**:267-277.
78. McClung, L. S., and McCoy, E., 1941. The anaerobic bacteria and their activities in nature and disease: A subject bibliography. Supplement one: Literature for 1938 and 1939. Univ. of Calif. Press.
79. McCoy, E., and McClung, L. S., 1938. Serological relations among the spore-forming anaerobic bacteria. Bact. Rev., **2**:47-97.
80. McCoy, E., and McClung, L. S., 1939. The anaerobic bacteria and their activities in nature and disease: A subject bibliography (in two volumes). Univ. of Calif. Press.
81. Macfarlane, R. G., Oakley, C. L., and Anderson, C. G., 1941. Hemolysis and the production of opalescence in serum and lecithovitellin by the toxin of *Clostridium welchii*. J. Path. and Bact., **52**:99-103.
82. McIntosh, J., and Selbie, F. R., 1941. Chemotherapy of gas gangrene. Lancet (London), **1**:240-242.
83. Maclean, I. H., and Holt, L. B., 1940. Combined immunization with tetanus toxoid and T.A.B. Lancet (London), **2**:581-583.
84. Marshall, M. S., Gunnison, J. B., and Luxen, M. P., 1940. Test for the sterility of biologic products. Soc. Expt. Biol. and Med. Proc., **44**:672-673.
85. Marshall, M. S., and Nordby, H. P., 1942. Anaerobic plates. J. Bact., **44**:619.
86. Marvel, D. M., and Parish, H. J., 1940. Tetanus prophylaxis and circulating antitoxin in men and women. Brit. Med. J., **2**:895.
87. Medical Research Council War Memo. No. 2, 1943. Notes on the diagnosis and treatment of gas gangrene. H. M. Stationery Office, London, 2nd edition.
88. Meleney, F. L., 1942. The important anaerobic infections and the use of zinc peroxide in their control. U. S. Naval Med. Bul., **40**:53-64.
89. Miles, A. A., 1940. The Rosenthal anaerobic method. Lancet (London), **2**:7.
90. Mollov, M., Winter, J. E., and Steinberg, P., 1942. Advantage of thioglycollate medium in routine diagnostic bacteriology. Amer. J. Clin. Path., **12**:571-579.
91. Mueller, J. H., and Miller, P. A., 1940. Tetanus toxin production on a simplified medium. Soc. Expt. Biol. and Med. Proc., **43**:389-390.
92. Mueller, J. H., and Miller, P. A., 1941a. Growth requirements of *Clostridium tetani*. J. Biol. Chem., **140**:933-934.
93. Mueller, J. H., and Miller, P. A., 1941b. A modification of Rosenthal's chromium-sulfuric acid method for anaerobic cultures. J. Bact., **41**:301-303.
94. Mueller, J. H., and Miller, P. A., 1942a. Folic acid in the growth of *Cl tetani*. Soc. Expt. Biol. and Med. Proc., **49**:211-212.
95. Mueller, J. H., and Miller, P. A., 1942b. Growth requirements of *Clostridium tetani*. J. Bact., **43**:763-772.
96. Mueller, J. H., Schoenbach, E. B., Jezukawicz, J. J., and Miller, P. A., 1943. Production of tetanus toxin on peptone-free media. J. Clin. Investigation, **22**:315-318.
97. Mueller, J. H., Seidmann, L. R., and Miller, P. A., 1943a. Antitoxin response in man to tetanus toxoids. J. Clin. Investigation, **22**:325-328.
98. Mueller, J. H., Seidman, L. R., and Miller, P. A., 1943b. A comparison of antigenicities of hydrolysate and peptone tetanus toxoids in the guinea pig. J. Clin. Investigation, **22**:321-324.

99. Nagler, F. P. O., 1939. Observations on a reaction between the lethal toxin of *Cl. welchii* (Type A) and human serum. Brit. J. Expt. Path., **20**:473-485.
100. Nagler, F. P. O., 1941. The estimation of the combining power of *Cl. welchii* (Type A) toxoid. J. Path. and Bact., **52**:105-110.
101. Newhouser, L. R., 1941. Tetanus toxoid immunization. Mil. Surg., **88**:371-374.
102. Nungester, W. J., Hood, M. N., and Warren, M. K., 1943. The use of thioglycollate media for testing disinfectants. J. Bact., **45**:44.
103. Oakley, C. L., and Warrock, G. H., 1941. Factors affecting the activity of the alpha toxin of *Cl. welchii*. J. Path. and Bact., **53**:335-370.
104. Orr, J. H., and Reed, G. B., 1940. Serological types of *Clostridium perfringens*. J. Bact., **40**:441-448.
105. Parish, H. J., and Oakley, C. L., 1940. Anaphylaxis after injection of tetanus toxoid. Brit. Med. J., **1**:294-295, 463.
106. Penfold, W. J., and Tolhurst, J. C., 1937. Formal toxoids in the prophylaxis of gas gangrene. Med. J. Australia, **1**:982-989.
107. Penfold, W. J., and Tolhurst, J. C., 1938. The prophylaxis of gas gangrene in man. Med. J. Australia, **1**:604-607.
108. Penfold, W. J., Tolhurst, J. C., and Wilson, D., 1941. Active immunization against gas gangrene and tetanus. J. Path. and Bact., **52**:187-201.
109. Peshkin, M. M., 1941a. Immunity to tetanus induced by combined alum-precipitated diphtheria and tetanus toxoids based on a study of one hundred and eighty-six allergic children. Am. J. Diseases Children, **62**:9-25.
110. Peshkin, M. M., 1941b. Immunity of tetanus induced by combined alum-precipitated diphtheria and tetanus toxoids based on a study of sixty-five allergic children given a third, or "repeat," dose. Am. J. Diseases Children, **62**:309-319.
111. Peterson, W. H., McDaniel, L. E., and McCoy, E., 1940. Biotin requirements of clostridia and assay of biological materials for biotin. J. Biol. Chem., **133**:lxxv-lxxvi.
112. Petrie, G. F., and Steabben, D., 1943. Specific identification of the chief pathogenic clostridia of gas gangrene. Brit. Med. J., p. 377-379.
113. Phillips, H. W., and McClung, L. S., 1943. Unpublished data.
114. Pike, C., 1940. Anaphylaxis after tetanus toxoid. Brit. Med. J., **1**:546.
115. Rainsford, S. G., 1942. The preservation of Vi antigen in T.A.B.C. vaccine with a note on combined active immunization with T.A.B.C. vaccine in tetanus formol-toxoid. J. Hyg. (London), **42**:297-322.
116. Ramon, G., 1939. Mixed vaccinations. New Internatl. Clin., Ser. 2, **1**:241-255.
117. Ramon, G., 1940. Combined (active-passive) prophylaxis and treatment of diphtheria or tetanus. J. Am. Med. Assoc., **114**:2366-2368.
118. Reed, G. B., and Orr, J. H., 1941. Rapid identification of gas gangrene anaerobes. War Med., **1**:493-510.
119. Reed, G. B., and Orr, J. H., 1942a. Local chemotherapy of experimental gas gangrene. War Med., **2**:59-78.
120. Reed, G. B., and Orr, J. H., 1942b. Treatment of experimental gas gangrene with zinc peroxide. War Med., **2**:79-82.
121. Robinson, C. L., and Stovall, W. D., 1939. A clinical bacteriological test for the recognition of *C. welchii* in wounds. Am. J. Clin. Path., Technical Supplement, **9**:27-32.
122. Rosenthal, L., 1937. "Chromium-sulfuric acid" method for anaerobic cultures. J. Bact., **34**:317-320.
123. Sachs, H., 1940. Anaphylaxis after injection of tetanus toxoid. Brit. Med. J., **1**:588.
124. Savill, A., 1940. Diagnosis of gas gangrene. Brit. Med. J., **1**:458-459.
125. Schaeffer, M., and Myers, G. B., 1940-41. Tetanus in a serum-sensitive patient successfully treated with a new despeciated antiserum. J. Allergy, **12**:188-190.
126. Schoenbach, E. B., Jezukawicz, J. J., and Mueller, J. H., 1943. Conversion of hydrolysate tetanus toxin to toxoid. J. Clin. Investigation, **22**:319-320.

127. Seal, S. C., 1942. A note on the production of *Clostridium perfringens* (Type A) toxin in a modified liver and veal digest medium. Indian J. Med. Research, **30**:229-243.
128. Seal, S. C., and Stewart, S. E., 1941. The application of the human serum opacity reaction for evaluating the antitoxin binding power (Lb) of *Clostridium perfringens* (Type A) toxoid. U. S. Public Health Reports, **56**:792-799.
129. Seiffert, G., 1939. Eine Reaktion menschlicher Sera mit Perfringenstoxin. Ztschr. f. Immunitätsforsch. u. Exper. Therap., **96**:515-520.
130. Sewell, R. L., 1940. Treatment of gas gangrene. Lancet (London), **1**:900-901.
131. Sewell, R. L., Dowdy, A. H., and Vincent, J. G., 1942. Chemotherapy and roentgen radiation in *Clostridium welchii* infections. Clinical and experimental studies. Surg., Gynecol., and Obstet., **74**:361-367.
132. Simmons, J. S., 1941a. Immunization against infectious diseases in the United States Army. South. Med. J., **34**:62-67.
133. Simmons, J. S., 1941b. Immunization against infectious diseases in the United States Army. Army Med. Bul., **57**:69-80.
134. Singer, E., 1940a. Experimental studies on the combined sulphanilamide and serum treatment of gas gangrene infections. Med. J. Aust., **2**:275-279.
135. Singer, E., 1940b. A note on the treatment of gas gangrene with sulphanilamide and related compounds. Med. J. Aust., **1**:796-798.
136. Singer, E., 1942. Studies on the value of x-ray therapy in experimental gas gangrene infection. Med. J. Aust., **2**:1-3.
137. Smith, W. H., 1942. The treatment of gas gangrene. Med. J. Aust., **1**:100-102.
138. Spooner, E. T. C., 1941. The bacteriology of air-raid wounds examined within forty-eight hours of infliction. Brit. Med. J., **2**:477-478.
139. Spray, R. S., 1930. An improved anaerobic culture dish. J. Lab. and Clin. Med., **16**:203-206.
140. Spray, R. S., 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. J. Bact., **32**:135-155.
141. Stephenson, D., and Ross, H. E., 1940. The chemotherapy of *Cl. welchii* Type A and *Cl. septique* infections in mice. Brit. Med. J., **1**:471-475.
142. Stewart, S. E., 1942. Antigenic value of *Clostridium perfringens (Clostridium welchii)* toxoid in prevention of gas gangrene. War Med., **2**:87-98.
143. Stewart, S. E., 1943. Active immunization of human beings with combined *Clostridium perfringens* and tetanus toxoids. War Med., **3**:508-511.
144. Strenge, H. B., 1940. Immunization with tetanus toxoid. The recall response. Yale J. Biol. and Med., **12**:487-491.
145. Tamura, J. T., Tytell, A. A., Boyd, M. J., and Logan, M. A., 1941a. Production of *Clostridium welchii* toxin in a peptone-free medium. J. Bact., **42**:148.
146. Tamura, J. T., Tytell, A. A., Boyd, M. J., and Logan, M. A., 1941b. Production of *Cl. welchii* toxin in peptone-free medium. Soc. Expt. Biol. and Med. Proc., **47**:284-287.
147. Taylor, A. W., and Stewart, J., 1941. The toxins produced by *Clostridium welchii* in a simple medium. J. Path. and Bact., **53**:87-94.
148. Unger, L., 1942. Tetanus toxoid in the prevention of tetanus and serum reactions in allergic individuals. Ill. Med. J., **81**:139-144.
149. Vera, H. D., 1942. Rapid demonstration of hemolysis due to anaerobes. J. Bact., **44**:507-508.
150. Weed, L. A., Echternacht, A. P., Meister, E. J., and Isenhour, R., 1942. The effect of therapeutic doses of x-ray on infections and inflammations. Experimental studies. Surg. Gynecol. and Obstet., **75**:157-160.
151. Weed, L. A., Forney, R., and Carter, E., 1942. Mechanism of the reaction between lecithovitellin and the toxin of *Clostridium welchii*. War Med., **2**:960-966.
152. Weed, L. A., and Minton, S., 1943. The nonspecificity of the serum opacity test for *Clostridium welchii*. J. Lab. and Clin. Med., **28**:1251-1253.

153. Weed, L. A., Minton, S., and Carter, E., 1942. Specificity of the lecithovitellin reaction in the diagnosis of gas gangrene due to *Clostridium welchii*. War Med., **2**:952-959.
154. Weinberg, M., and Barotte, J., 1928. Synergie des anticorps. Ann. Inst. Pasteur., **42**:619-641.
155. Weinberg, M., Nativelle, R., and Právot, A. R., 1937. Les microbes anaerobies. Masson et Cie., Paris.
156. Welch, H., Slocum, G. G., and Herwick, R. P., 1942. Tetanus from sulfonamide dusting powders. Development in guinea pigs treated with sulfonalamide powder contaminated with tetanus spores. J. Am. Med. Assoc., **120**:361-364.
157. West, R. A., Lewis, K. H., and Militzer, W. E., 1940. Growth of some nonsporulating anaerobic bacteria in synthetic media and extracts from corn silk. J. Bact., **40**:332.
158. West, R. A., Lewis, K. H., and Militzer, W. E., 1941. Growth of nonsporulating anaerobic bacteria of intestinal origin in synthetic media. J. Bact., **41**:42-43.
159. Whittingham, H. E., 1940. Anaphylaxis following administration of tetanus toxoid. Brit. Med. J., **1**:292-293.
160. Wilsdon, A. J., 1931. Observations on the classification of *Bacillus welchii*. Univ. Cambridge, Inst. Anim. Path., 2nd Report of the Director.
161. Wooley, D. W., McDaniel, L. E., and Peterson, W. H., 1939. Growth factors for bacteria. IX. Studies of a growth factor for clostridia. J. Biol. Chem., **131**:381-385.