SEROLOGICAL RELATIONS AMONG SPORE-FORMING ANAEROBIC BACTERIA

ELIZABETH McCOY AND L. S. McCLUNG

Department of Agricultural Bacteriology, University of Wisconsin, Madison, and George Williams Hooper Foundation for Medical Research, University of California, San Francisco

Received for publication March 2, 1938

Immunochemistry in recent years has offered new proofs and better criteria for the specificity of antigen-antibody reactions (134, 174, 175, 207). These advances have strengthened the position of serologists by providing a firmer basis for their examination of biological materials, such as bacterial constituents. Remarkable examples of group- and species-specificity have been found, and in certain instances the information has been useful in bacterial classification.

It is the purpose of this review to present and evaluate the serological data relating to the spore-forming anaerobes[1] and to discuss the relationships disclosed and their bearing upon the taxonomy of these organisms. One must admit, however, that the serological relationships are not always in harmony with the present taxonomic schemes. Examples will be given in which the serological evidence may require consolidation of species that are at present listed separately. In other instances, the evidence either confirms the present species or reveals subdivision of them. For the present, then, it can only be claimed that the serological approach has discovered a certain measure of order, and that it does aid in the differentiation of the closely related spore-forming anaerobes.

The subject-matter of this review will be discussed under the

[1] Comparatively little is known concerning the serology of the non-spore-forming species, although several papers on this topic have appeared lately: 9–13, 59–61, 79, 80, 145, 303, and 364.

headings: *a* Toxigenic anaerobes and their toxin specificities (including the problem of atoxic strains); *b* Agglutination reactions; and *c* Precipitin and complement fixation reactions.

THE TOXIGENIC ANAEROBES AND THEIR TOXIN SPECIFICITIES

The toxins produced by certain of the spore-forming anaerobes have long been recognized as specific substances, though of unknown composition. Physiological evidence of this specificity is found in the consistent action in the animal body of such toxins as tetanospasmin and the neurotoxin of *Clostridium botulinum*.[2] The specificity of the toxin-antitoxin reaction was employed in the recognition of an anaerobe by Roux as early as 1888 (271). Through the World War period, when discovery of new anaerobes was abnormally stimulated, it became almost a routine to test for toxin neutralization with antitoxins of the most probable group relatives before a newly isolated strain was identified or described as new (52, 55, 168, 200, 277, 359, 360). In polymicrobial infections, like gas gangrene, proof of the etiologic agent was often obtained by "animal protection experiments" (70, 144, 176, 177, 263, 330). And finally, the controversy as to whether "Rauschbrand" of animals was comparable to gas edema in man, and caused by the same organism, was settled largely by study of the toxin specificities of *Clostridium chauvoei* and *Clostridium septicum* (55, 107, 169, 178).

Partly because the toxins are usually produced in complex nitrogenous media, the problems of the purification and nature of these substances are difficult to solve. Nevertheless, considerable efforts are being made to develop methods for purifying,

[2] Throughout this paper problems of nomenclature have been ignored so far as possible. In general, the name first proposed has been employed in direct reference to the discovery of an organism; in section headings and in later discussion that name, as adapted to placement in the genus *Clostridium*, has been used for all commonly accepted members of the genus. For the less familiar *Clostridium* species and particularly those whose familiar names have thus been displaced, synonyms have been given in parentheses following the first citation of the species. Whenever inclusion of an organism in *Clostridium* would involve the creation of a combination of names new to the literature, the transfer has not been made; in such cases, the original name in *Bacillus* has been retained for clarity of reference. This policy has seemed to the authors preferable to debating and deciding specific problems of nomenclature on this occasion.

preserving, and characterizing the principal toxins and antitoxins (19–24, 33, 78, 101–104, 129, 148, 152–154, 158, 204, 205, 290, 293, 298–300, 312, 313, 323, 336, 337, 339, 371). Recently two agencies, the Inter-governmental Conference on Biological Standardization and the Permanent Standards Commission of the League of Nations Health Organization, have undertaken to define international units for toxin and antitoxin preparations for tetanus and the gas gangrene organisms (1, 93, 94, 152, 153, 193, 204, 205, 337). The work of the League Committee has been directed primarily toward the definition of units and the investigation of the accuracy of titrations of toxin-antitoxin preparations. The State Serum Institute at Copenhagen is the depository for the standards. As the United States is not a member of the League, it has no official part in determining the international policy; but it has coöperated in conferences and in making tests. Definitions of the United States equivalents of the International units are made by the National Institute of Health. In some cases, the same units have been adopted, for example, that for oedematiens antitoxin (23); in other cases, a pre-existing American unit has been defined in terms of the International unit, for example, that for tetanus antitoxin (193).

Enough is known about the differentiation of the toxins of the principal anaerobic species to permit a discussion of these specific substances as a basis for the typing of the toxigenic organisms.

The toxins of Clostridium tetani, Clostridium septicum, and Clostridium histolyticum

Clostridium tetani presents a comparatively simple case of toxigenicity. Its toxin was discovered by Kitasato in 1889 (166), and the specific antitoxin by von Behring and Kitasato in the following year (15). The pharmacological action of the toxin indicates the presence of at least two factors: tetanospasmin and tetanolysin (87, 118, 181, 203, 258, 326, 335). For purposes of production of antitoxin, however, no distinction of these factors is made, the cell-free filtrate of a culture being used either directly, or modified by iodine (329), formol (278), or in combination with alum, tapioca, lanolin, etc. (255–257). Furthermore, experience has shown that a protective serum can be made

from any strain of true *C. tetani.* For this reason the toxin is called monotypic, a point of particular interest in view of the subdivision of the species into serological types by other reactions. The question of the mode of absorption and action of tetanus toxin has recently been reopened; but there is not as yet agreement upon the route of transfer of the toxin to the central nervous system (2–6, 74, 90).

Clostridium septicum (*Vibrion septique; Bacillus oedematis maligni*) also produces a toxin which has no type-specificity, although sub-types on the basis of agglutination do exist within the species. The general serological properties of the toxin are known (19, 20, 71, 158, 264, 266, 338, 348). The pathology of the extremely rapid death of animals injected intravenously with the toxin has been explained recently (245). A specific action of the toxin on the heart muscle, causing Zenker's degeneration, is the immediate cause of death, although lesions also occur in the kidney, spleen, and other organs.

Clostridium histolyticum also produces a toxin which is monotypic for the species but is apparently a complex of myolytic and hemolytic factors. The organism is unique in its myolytic action, a property which has been made use of in the Connell method (54) for the lysis of cancerous tissue. The efficacy of this treatment has been denied (117, 250, 272); but its proposal has at least led to a more thorough study of the proteolytic enzyme system, upon which the myolytic action probably depends (309, 340). It is peculiar that the red blood corpuscles remain intact in the residue of lysed tissue (123, 362). In culture, hemolysin is produced under proper conditions, but not in proportion to the general potency of the toxin as determined by the minimal lethal dose (228, 309). The preparation of the toxin and antitoxin and their standardization have recently been investigated (24, 153, 337).

The toxin of Clostridium oedematoides (*Clostridium oedematis sporogenes; Bacillus sordellii*)

A new species of the gas-gangrene group was discovered by Sordelli in 1922 (301). Because of its resemblance to *Clos-*

tridium oedematiens in pathogenicity in a human case and to *Clostridium sporogenes* in culture, it was called *Clostridium oedematis sporogenes*. The name, being a trinomial, was objectionable and was changed to *Bacillus sordellii* by Hall and Scott (127). Meanwhile, Meleney, Humphreys and Carp (219) discovered a *Clostridium oedematoides*, resembling *C. oedematiens* and *C. septicum* but distinguishable from both by toxin neutralization tests. The following year the toxins of *C. oedematoides* and *B. sordellii* were found to be identical; *C. oedematoides* was declared invalid and *B. sordellii* considered the preferred name for the species (125, 150). Agglutination tests have also indicated that the two organisms are identical (126). However, since *Clostridium* is now the widely accepted genus for the spore-forming anaerobes, *C. oedematoides* would be valid, and it has been retained by Hauduroy *et al.* (131) in the new French "*Dictionnaire des bactéries pathogènes pour l'homme, les animaux et les plantes.*" Nevertheless, the combination: *B. sordellii* or *Clostridium sordellii*, according to the choice of the user, continues to be in favor in this country.

Further study of the toxin-antitoxin reaction of this species has disclosed an unforeseen relationship to another anaerobic species. In the course of their study of *Bacillus bifermentans*, Clark and Hall (47) discovered positive but "weak" cross-agglutination between *B. bifermentans* and *B. sordellii*. Moreover, the serum of rabbits immunized with *B. bifermentans* was found to be protective to guinea pigs injected with toxin of *B. sordellii*. However, since the pathogenicity of *B. sordellii* contrasts sharply with the non-pathogenicity of *B. bifermentans*, Clark and Hall did not recommend combining these species. Spray (304) has also respected the difference in pathogenicity and has made it the only point of separation in his key to the spore-forming anaerobes. The relationship has been further investigated by Stewart (310), who has found 1 cc. of an experimental antiserum for *C. bifermentans* to protect mice against 2 to 5 M.L.D. of the toxin of *C. sordellii*. Positive agglutination and precipitation reactions have also been obtained, thus linking the two species. Because they are also indistinguishable in

morphology, colony formation, and biochemical reactions, consolidation has been recommended under the name: *C. bifermentans*, which has priority over *C. sordellii*. The antitoxin would then be called by Stewart bifermentans antitoxin, but whether this terminology will come into general use remains to be seen. A somewhat analogous case involving *Bacillus oedematis maligni* II of Novy (now called *Clostridium novyi*) and *C. oedematiens* will be discussed later.

Ictero-hemoglobinuria of cattle in Nevada is caused by an organism which was at first called *Bacillus hemolyticus bovis* and later, *B. hemolyticus* (331, 333). For a time, there was some question whether it also could be identified with *C. oedematoides*, but apparently the latter is merely associated with it in certain cases of the disease (120).

The toxins of Clostridium parabotulinum and Clostridium botulinum[3]

After recognition by Leuchs (182) of the production of multiple toxins by *Clostridium botulinum*, Burke (45) reported two distinct toxin groups, arbitrarily called Types A and B, in the 12 American strains studied by her. The antitoxin of one type neutralizes the toxin of the homologous but not of the heterologous type. An important contribution by Bengtson (16, 18) revealed a third toxin group, Type C, represented by cultures[4] from larvae of *Lucilia caesar* taken from a chicken, which had

[3] The nomenclature of the botulinus-parabotulinus group is particularly confusing, because of a lack of understanding of their separation on the basis of proteolysis. The terminology was introduced by Bengtson in 1924 (18). As recently as 1937, however, the distinction between *C. botulinum* and *C. parabotulinum* was not appreciated, which unfortunately adds to the confusion (369). It is beyond the scope of this review to argue the nomenclature; the original basis of separation and discussions regarding it may be found in the following articles: 18; 112; 131; 186; 221–224; 268; 288; 315–319; 321, pp. 687–690, 1276–1279; 350, p. 107; 358, pp. 332–340; 369. We have accepted the terminology of Bengtson and have therefore used *C. botulinum* to refer to non-proteolytic cultures found within the toxin types B, C, D, and E. Proteolytic cultures, termed *C. parabotulinum*, have been found thus far only within the toxin types A and B.

[4] The Type C organism has been called *Clostridium luciliae* by Bergey (29) and by Spray (304), but the name has never come into common use and seems not likely to, because it is at variance with the Bengtson nomenclature.

died of limberneck. Probably identical is the organism later isolated by Graham and Boughton (106), and in the same toxin group, at least, is the organism of Seddon (287, 288) from botulism of cattle in Tasmania. Pfenninger (248) showed that the Seddon organism produces a toxin, which can be neutralized by the Type C antitoxin prepared by Bengtson, but that the antitoxin for the Seddon culture neutralizes only its homologous toxin. These observations have been confirmed, and the two factors have been designated C_α and C_β, within Type C toxin (110, 112, 114). A Type D toxin was added after study of the organism associated with botulism or lamsiekte of cattle in South Africa (224, 315, 316, 319). Finally, a Type E was proposed by Gunnison, Cummings and Meyer (111) to designate the toxin of an organism obtained from Russia and originally isolated from spoiled fish. (Topley and Wilson (321, p. 689) have erroneously cited Type E as the cause of equine botulism in South Africa; in reality (268), the disease is due to *C. botulinum*, Type C.) Apparently the organism of Hazen, which was unfortunately incompletely reported (132), is also a Type E culture.[5] Thus, there are now five types of the botulinus toxin.

The work of Mason and Robinson (216) is of interest because of its extensive experiments on the antigenic components of these toxins. The A and B toxins appear to be monospecific, whereas the C and D toxins are not so simply constituted. It has been claimed that C toxin is composed of two major fractions, C_1 and C_2, which may vary quantitatively with the conditions of incubation; and that D toxin is present, but in small amount only, in the so-called Type C toxin. Although the Type D organism produces chiefly D toxin, there is also apparently some admixture of C toxin. Confirmation of this distribution of the fractions is needed; and extended study should be made of all available cultures of these types, with particular attention to the conditions for production of the toxins. The new Type E strains and others, which may be isolated from widely separated geographical regions, should be included in the study.

[5] Personal communication from Dr. K. F. Meyer. A paper reporting this finding is in preparation.

The toxins of the Clostridium welchii group

From the time of isolation of the organism now called *Clostridium welchii* (*Bacillus aerogenes capsulatus, B. phlegmonis emphysematosae, B. perfringens*) there have been many reports of its remarkable pathogenic action. The acid (principally the butyric acid) was at first regarded as a tissue irritant and debilitant (49). As late as 1916, it was argued that no true toxin was concerned but that the acid injury was a sufficient cause of the lesions of gas gangrene (307). The mechanical effect of the gas in blocking circulation and contributing to the spread of infection was also considered important (202). The severe toxemia and the rapidly fatal outcome, however, made it seem likely that a potent toxin was involved. In 1917, Bull and Pritchett (42–44) obtained the soluble toxin *in vitro* and prepared the antitoxin. The main properties of the toxin were soon determined; and again a multiplicity of toxin elements was indicated. These have been named after their apparent physiological action: a hemotoxin or hemolysin (42, 46, 88, 142, 157, 242, 280, 357, 374); a myotoxin (143); a neurotoxin (346); another toxin said to act specifically on the blood vessels (347); "necrotic and lethal" toxin (99); the "acute lethal" toxin of Shiraishi (289); and finally the so-called "pseudotoxin" (7, 170), which is heat-stable, non-antigenic, and non-neutralizable by antitoxin. This "pseudotoxin" may be the histamine or histamine-like substances found among the growth-products of *C. welchii* (162). With such an array of toxin elements (though some of them appear to have been proposed on very meagre evidence), it is no wonder that the testing of toxin and antitoxin of *C. welchii* presents considerable difficulty. The proportion of hemolysin, for example, varies widely (357), and this variation interferes with the measurement of the protective power of an antiserum by the titration of its antihemolysin content (99, 143, 215, 252, 253, 354).

Further evidence of the complexity of *C. welchii* toxins appears, when one considers the several organisms resembling, but apparently not identical with, the *C. welchii* of classical gas gangrene. In recent years many reports have claimed, or questioned, the association of *C. welchii* with intestinal disorders of man, such as

flatulent diarrhea (161, 234, 311), or the toxemia of intestinal obstruction (146, 201, 233, 320, 366). Also, in diseases of the enterotoxemic type among domestic animals, there are found organisms resembling *C. welchii*. Lamb dysentery in Great Britain, one of the first of the enterotoxemias to be investigated, is ascribed to the lamb dysentery bacillus of Dalling (62, 92), sometimes called *Bacillus agni*. The bacillus is not identified as *C. welchii* because of minor physiological differences, and because the antitoxin for *C. welchii* is not capable of neutralizing the toxin of the Dalling bacillus, although its antitoxin is capable of neutralizing the toxin of *C. welchii*. However, the hemolysins of the two organisms can be neutralized by either antitoxin. Soon another organism from a sheep disease, called *struck* in England, was discovered by McEwen (195–198). It resembles both *C. welchii* and the Dalling bacillus, but differs in a few physiological reactions, and significantly in its toxin-antitoxin specificity. The toxin of McEwen's organism, *Bacillus paludis*, is not neutralized by the antitoxin of *C. welchii* (human), nor the toxin of *C. welchii* by the antitoxin of *B. paludis*. McEwen inferred, therefore that his organism differed also from Dalling's bacillus, especially since he was not at first able to show that *B. paludis* produces a hemotoxin. Complicating still further this group of *welchii*-like organisms of animal diseases is the organism found in enterotoxemia of sheep in West Australia (25, 27) and the "pulpy kidney" disease of sheep reported from New Zealand (97, 98), Tasmania (26, 244), Australia (26), Palestine (96), and North Wales (229, 230). Apparently the same organism occurs in other herbivorous animals, and it has been considered as the possible etiologic agent in the grass disease of horses (105). Bennetts (25) discovered and described the organism of sheep enterotoxemia in 1932. Again, because of the differential specificity of its toxin, as evidenced in cross-protection experiments with the classical *C. welchii*, *B. paludis* of McEwen, and the Dalling bacillus, Bennetts considered his organism a new species, which he named *Bacillus ovitoxicus*.

Prophylaxis of the various sheep diseases has been tried with both antitoxin and toxoid vaccinations (27, 68, 69, 212). In

experiments of this nature, it is important that the cross-protective powers of the antisera produced for the several species be known, because investigation of the distribution of the diseases has shown that the organisms may occur widely and at random, —*B. paludis* has been reported in France, Greece and Turkey; *B. ovitoxicus* in Scotland, North Wales, Palestine, United States, etc. (25, 27, 64, 96, 231, 355, 356). Studies of the toxin-antitoxin relations were made at the Cambridge Institute of Animal Pathology and the Wellcome Physiological Research Laboratories at nearly the same time. The Wilsdon report from Cambridge was published first, showing clearly four organisms, Types A, B, C, and D, on the basis of toxin-antitoxin reactions (367). Type D was then new in England and, although subsequently shown to be *B. ovitoxicus* Bennetts (368), it is still often called the Wilsdon Type D organism. The toxins and antitoxins of these organisms display considerable overlapping of factors, the details of which as determined by cross-protection experiments are as follows:

ORGANISM	TOXIN TYPE	TOXIN FACTORS	ANTITOXIN NEUTRALIZES
Cl. welchii	A	W	A
Dalling's	B	WXZ	A, B, C, and D
B. paludis	C	WZ	A, B, and C
B. ovitoxicus	D	WX	A and D

Wilsdon closes his report with the statement that "although the strains can also be grouped according to one or another of their biochemical characters, it is held that these are of minor importance compared with differences in antigenic structure revealed by toxin-antitoxin reactions."

In 1933, Glenny, Barr, Llewellyn-Jones, Dalling and Ross (100) of the Wellcome Laboratories announced the toxin fractions α, β, γ, and δ; to these was added the factor ϵ, said to be present in Dalling's early cultures of the lamb dysentery bacillus but weak or lacking in many Type B strains of the present day (211). Prigge (252–254) has recently added a ζ factor, so far

known only in the true *C. welchii* or Type A. The properties of these factors in physiological terms are (65, 211, 252):

α—hemolytic, lethal and necrotic
β—lethal and necrotic
γ—lethal
δ—hemolytic
ϵ—lethal and necrotic
ζ—lethal

The distribution of these factors in the respective toxin types is as follows (65):

TOXIN TYPE	FACTORS					
	α	β	γ	δ	ϵ	ζ
A	+	−	−	−	−	+
B	+	+	+	±	+	
C	+	+	+	+	−	
D	+	−	−	−	+	

With six factors involved in the toxin complex, instead of the three (WXZ) originally recognized by Wilsdon, problems of cross-protection for the four types of *C. welchii* organisms will require reinvestigation, the more so since five of the six factors are reported to be lethal. Nicholson (235) studied the action of the whole toxins of the Type A, B, C, and D organisms on the circulatory and respiratory systems; but his findings are difficult to interpret for obvious reasons.

The most remarkable organism of the four is Type B, or Dalling's lamb dysentery bacillus, which has at least five of the toxin factors. Its antiserum should therefore protect against the toxins of Types B, C, and D. The discrepancy to be explained is that it also protects against Type A toxin, according to Wilsdon and Montgomerie and Rowlands (232, 367), although the lethal ζ toxin of Prigge is not known to be formed by Dalling's bacillus. Similarly Type C antiserum, which should lack ϵ antitoxin, protects against Type B toxin, which has the ϵ factor. That there is still some fine point of relationship to be worked out between

these two types is probable, because Dalling's laboratory has record of a Type B culture, which has become Type C by loss of the ϵ factor (64). The classical *C. welchii* would seem to be the most restricted of all in respect to toxin factors, yet there is still something to be explained in the cross-protective power of its antiserum. Commercial antisera for *C. welchii* are of Type A mono-specificity, as judged by their neutralizing power. Nevertheless, they will protect guinea pigs from infection by Type D organisms, which differ from the Type A organisms in possessing the ϵ toxin factor (31, 32).

The suggestion has been made (36) that gangrene antiserum for man might be improved by being made polyvalent, for apparently there is ample opportunity for infection of man by any member of the *C. welchii* group. Thus far, however, Types B, C, and D are known as pathogens of domestic animals only; but they can upon parenteral injection give rise to typical gas gangrene in experimental animals (197a). Very little is known of the distribution of the types of *C. welchii* in the intestinal tracts of man and other animals. Borthwick and Gray (35) have evidence of only Type A in human feces, whether of normal individuals or of a patient with pernicious anemia. In rabbits only Type A has been found and in guinea pigs only Type D (34). In the dog both Types A and D were found, but investigation showed that the newly-isolated Type D strains tended to revert to Type A, which involves only loss of their X factor (Wilsdon's terminology). A further study of the D→A conversion would be of decided interest, in view of Borthwick's finding (31) that commercial mono-A type sera will protect experimental animals from either Type A or D infections, although neutralizing only Type A toxin. Prigge's last contribution (254) on the importance of the ζ toxin of Type A organisms in relation to the protective power of the sera, should be considered also in the problem of the relation between Types A and D organisms. For the maximum protective power of *C. welchii* antisera, Weinberg (341, 352, 353) has advocated those sera which are both antitoxic and antibacterial. Such sera he would call "holosera" or "anti-

exo-endo-toxic sera." Their advantage has not yet been adequately shown.

Other papers have appeared characterizing the interrelations of these organisms and have confirmed the main facts (31, 72, 122, 211, 217). In one case, cultures derived from single cells were used to dispose of the possibility that the overlapping of toxins and certain irregular proteolytic action of the types might be due to mixture of the closely related organisms (210).

Such is the present state of knowledge of the toxins of the *C. welchii* group. Some points of uncertainty remain: whether the lamb dysentery strain of 1923 possessed the toxin factor for *B. ovitoxicus*, as its serum seemed to indicate (63); why there are discrepancies in hemolysin production by cultures tested at different times (195, 252, 354); how stable the toxin types are, in view of the D→A conversion, and so on. The etiologic agents of sheep diseases in different parts of the world are still being investigated. Recently, for example, the "bloedpens" of South Africa has been identified with the lamb dysentery of Great Britain, and its causal organism also found to be *C. welchii* Type B, complete with the ϵ toxin factor (211, 213). Lamb dysentery in Montana is apparently not the same (208). Enterotoxemic jaundice or sheep yellows in Australia is due to *C. welchii* Type A (269). The peculiarities of the enterotoxemias are not yet fully understood. There is an intestinal factor, possibly trypsin, obtained in filtrates of the intestinal contents, which when added to Type D toxin renders the mixture very much more toxic to the mouse (37, 38). A "diffusion factor" in cultures of various anaerobes, including *C. welchii*, has also been reported (184). Until more is known of these factors which apparently affect the permeability of the intestinal walls, it is difficult to evaluate the reports. They are perhaps important in determining the pathogenicity of the *C. welchii* regularly found in the intestinal tract.

Short reviews of the interrelations of the toxins and of the etiology of the various diseases due to the *C. welchii* group are available (64, 72, 373). Discussions at scientific meetings have brought out interesting points, some of which have not been made in other published papers (32, 36, 65–67, 286, 342, 343).

The toxin of Clostridium oedematiens and its near relatives

Clostridium oedematiens was found by Weinberg and Séguin in certain cases of gas gangrene, occasionally alone but more often associated with *C. welchii*. Its toxicity was proven and it was successfully differentiated from *C. septicum* and *C. welchii* (359, 363). The therapeutic value of the *C. oedematiens* antitoxin has justified its inclusion in the polyvalent sera for gas gangrene (94, 204). Fortunately, the toxin is monotypic, for Weinberg, Nativelle, and Prévot (358) have stated that the antisera will neutralize toxin of all strains, whether of human or animal origin. Toxigenicity is relatively weak, or appears to be so because of the instability of the toxin. The properties of the toxin and the conditions for its preservation have recently been investigated (23, 339).

There remains, however, the question of separation of the toxin of *C. oedematiens* from those of certain of its close relatives. Sacquépée (275, 277) discovered an organism at first called "bacille de l'oedème malin," later *Bacillus bellonensis*. There was for a time doubt of its purity and of its relation to *C. oedematiens* (274, 276, 361), but it has recently been accepted by Weinberg (358) as a separate species with a specific toxin. Sacquépée (277) distinguished it from *C. septicum* by toxin-antitoxin reactions.

Another organism very close to *C. oedematiens* is the *Bacillus oedematis maligni* II of Novy (now called *Clostridium novyi*). It is often considered synonymous, but Weinberg regards it as an atoxic variety of his organism, remarking that it would probably have been so recognized had it been discovered *after*, instead of *before*, *C. oedematiens* (358).

Bacillus gigas Zeissler and Rassfeld (380) is also similar to *C. oedematiens*. It was found in a disease of sheep, resembling bradsot or braxy, and was considered different from *C. oedematiens* principally because of the conspicuous size of its cells (1 to 2 by 4 to 20 micra) and because of certain cultural differences. It produces a toxin, whose relation to the toxin of *C. oedematiens* has apparently not been explored. Kraneveld, who found *B. gigas* in diseased animals in the Dutch East Indies, also reported

another organism (172), the bacillus of *osteomyelitis bacillosa bubalorum* of the Indian water buffalo; he later identified his organism with the "Novy group" as defined by Miessner, Meyn, and Schoop (173, 227). None of the descriptions of *B. gigas* is as complete as one would wish, and further study of its toxin is needed. The same may be said of other oedematiens-like organisms, which have been found in animal diseases: bradsot and braxy, infectious necrotic hepatitis, black disease, and big head of rams. These and probably others of the so-called braxy group are not as yet well characterized (with a few notable exceptions, *e.g.*, black disease (243, 328)). Perhaps in time a complexity of causal organisms like that of the *C. welchii* group will be found, and if so, it is not improbable that knowledge of the toxin specificities of the group will aid in its resolution.

The toxin of Clostridium chauvoei

Clostridium chauvoei should perhaps be considered above, close to *C. septicum*, for it is of that general affinity. We choose to discuss it here, however, because for a time there was debate as to whether it formed a toxin, and certainly there are strains which have not been shown to do so in the laboratory. It thus represents, in a sense, a transitional organism to the non-toxigenic forms, and will serve to introduce mention of "non-toxic or atoxic" strains of supposedly toxigenic species.

C. chauvoei is the cause of blackleg, symptomatic anthrax, quarter evil, or "Rauschbrand," as the disease is called in different localities. Production of toxin was claimed by several early workers (151, 159, 171, 179, 236, 238) but denied by Scott and others as late as 1928 (130, 283–285). Recent work, however, has shown that toxin can be elaborated under proper conditions of culture (165, 214). The explanation of the disagreement seems to be that toxigenicity is easily lost, and consequently non-toxic strains are more generally encountered in this species than in other common anaerobes (8, 66, 130, 151, 167, 179). Perhaps because of the instability of the toxigenicity, more than usual effort has been made to practice active immunization against this disease. Many types of blackleg prophylactic products have

been tried: filtrates of cultures (at times probably toxin-containing), vaccines of various kinds, anacultures, even infected muscle powder and spore vaccines. These products may carry also lysins; and the immunity which they induce is undoubtedly complex. Hence it is very difficult to assess either the importance of the antitoxic factor in the immunity produced, or to claim for it any conspicuous laboratory usefulness in diagnosis or typing. As will be seen below, agglutination, precipitation, and complement fixation may be resorted to in the classification of such organisms.

The problem of atoxic strains

It must be emphasized in passing that *C. chauvoei* is not unique in possessing atoxic strains. The literature cites the following: *C. tetani* (53, 84–86, 89, 163, 164, 206, 262, 263, 326); *C. welchii* (34, 35, 144, 183, 199, 201, 239, 240, 262, 289, 291, 336); *C. botulinum* and *C. parabotulinum* (17, 18, 45, 76, 113, 216, 241, 248, 267, 268, 322, 334); *C. oedematiens* (23, 39, 48, 359); *C. hemolyticum* (332); and the bacillus II of Ghon and Sachs (314). In most of the above cases the atoxic strains were found at isolation, or they developed spontaneously during laboratory cultivation of the originally toxic cultures. Occasionally, cultivation to produce variants was intentionally undertaken.

Loss of toxigenicity often throws a variant extremely close to the borders of another species. Who is to say that an atoxic strain of *C. tetani* is not within the confines of *Clostridium tetanomorphum*, or an atoxic *C. welchii* within those of *C. butyricum*? This weakness of the method, or perhaps one should say of our conceptions of the species, can in some cases be corrected by correlation of other antigenic reactions, agglutination or precipitation or complement fixation. Reference will be made to the placing of atoxic strains of *C. tetani* and *C. parabotulinum* by such means. But at least the possibility of missing entirely the relations of a strain, because of its atoxicity, brings the realization that the toxin neutralization test is not even a generally applicable method for typing anaerobes, since the many nontoxic species are entirely beyond its reach. In general applica-

tion therefore, any method of analysis of the intact cellular antigens is to be preferred. In our opinion, agglutination, properly carried out, serves that purpose, and its usefulness will be discussed next.

THE AGGLUTINATION REACTION APPLIED TO SPORE-FORMING ANAEROBES

First recognized during a comparison of the blood of animals normal and immune to tetanus, agglutinins for anaerobes were found soon after the phenomenon of bacterial agglutination itself was known (56, 57, 273). Early application was made to the distinction of two anaerobic species, *C. chauvoei* and *C. septicum*, by Leclainche and Vallée (178). Since then the method has had extensive use, as we can testify, having read more than 250 papers on this one phase of the serology of anaerobes! There has not been complete satisfaction with the method; and it will perhaps best serve the present purpose to mention some of its difficulties and failures, as well as its usefulness.

The first hint of difficulty appeared in a paper by Nicolle and Trenel (237) on the variability of agglutinative aptitude and agglutinogenic function, in which it was stated that anaerobes are: "infiniment moins sensibles et. . . . souvent variables." Nevertheless, in the monographs on anaerobes after the World War (52, 75, 350, 378, 379) it was possible to list those organisms for which satisfactory agglutinating sera had been prepared, and the few for which they had not.

Although Meyer (200) had in 1915 recommended agglutination, Zeissler (376), Heller (135), and Hall (119) categorically condemned it as an aid in the typing of anaerobes. Hall and Stark (128) from their experience with *C. sporogenes*, however, stated that "one should regard serological agglutination as a satisfactory criterion of species identity in properly controlled positive tests, but not of differentiation in negative tests." One of the chief complaints concerned the instability or "auto-agglutinability" of the anaerobic cell suspensions used as antigens; for instance, "L'auto-agglutination des cultures du *B. oedematiens* en milieux liquides est une des caractéristiques de cet anaérobie. Il est

donc impossible d'étudier l'agglutination de ce germe en recourant aux procédés ordinaires." (345). It is unfortunate that the *Manual of Methods for Pure Culture Study of Bacteria* (41, 121) still recommends the use of broth cultures of anaerobes directly as antigen, either for injection or for macroscopic tube agglutination. Particularly in glucose broth, and some form of it is likely to be used with anaerobes, the acidity resulting from fermentation may cause flocculation of the cells. Heller (135) had the key to correction of this difficulty, when she secured stability by adjustment of the electrolyte concentration and the pH of the antigen suspensions. Snyder (294) recently re-emphasized the danger of non-specific agglutination by acid, and established a critical pH for the species with which he worked. If broth cultures are ever to be considered as antigens, it is important to know that the cells in broth may be flocculated at a pH distinctly higher than in distilled water. To avoid the errors due to autoagglutination, Plaut (249) devised a dark-field method for agglutination in hanging drops; he claims that true agglutination is easily detected by the loss of motility of the clumps.

Two other complaints, (a) that for certain groups of anaerobes it is very difficult or "impossible" to produce agglutinating sera, and (b) that for others the agglutination may be ultra-specific, can best be dealt with by presenting the pertinent data for the major groups of anaerobes.

But first we would point out that methods of agglutination have undergone considerable change from the first simple mixing of culture and serum. The Oxford method of achieving "standard agglutination" was a distinct advance. It was followed by the flagellar-somatic agglutination of motile organisms, evidence for at least a dual nature of the agglutinogens. This concept was shrewdly criticized by Tulloch (327), as it was then a purely qualitative theory, little more than the old mosaic hypothesis of Durham and Ehrlich. But there can now be no doubt that separate antigens do exist independently within the cell, as evidenced by the many isolations of protein and polysaccharide fractions. Furthermore, the apparent success in resolving the *Salmonella* group by analysis of their somatic and flagellar anti-

gens is an outstanding example. The "Salmonella Subcommittee of the Nomenclature Committee of the International Society of Microbiology" (279) has recently published its findings on the genus *Salmonella*, in a scheme based on the Kaufmann-White antigenic factors of the group. It is of interest also that the League of Nations Health Organization has in April, 1937, adopted a detailed technique to be used in the agglutinative diagnosis of enteric infections (82). These recent uses, and a review and interpretation of the techniques of agglutination by Cruickshank (58), are commended to the reader. For specific agglutination methods which have succeeded with anaerobes, the following papers may be consulted: 83, 109, 136, 190, 281.

The agglutination of Clostridium tetani

As mentioned above, *C. tetani* was the first of the anaerobes to reveal its agglutinative powers. However, nothing of importance concerning the typing of the species appeared until Tulloch (324–326) discovered a subdivision into five serologic types. On a combination of the evidence from several workers (14, 50, 51, 85) nine types are now known. In 1928, the flagellar-somatic method of agglutination was applied to *C. tetani* by Felix and Robertson (83), and the dual nature of the agglutinogens was found for the first time to be true for a motile anaerobe as well as for motile aerobes. The type-specificity of former groupings (only seven were then known) was shown to lie within the reactions of the flagellar or thermolabile H antigen. There was included in the study a so-called "pure O" strain,—a non-motile variant by chance devoid of the H antigen proper to its species. Parenthetically, it may be said that such strains, if available, are used to produce potent somatic antisera; otherwise, the H antigen of a motile strain may be destroyed by heat or chemicals and the resulting product used as the O antigen. Antiserum for the pure O strain of *C. tetani* was found to agglutinate in the "small flaking manner"; and from this it was concluded that "in the stable antigen the group relationship is very close indeed," in contrast to the type-specificity of the H antigen. With so-called "smooth" and "rough" strains of *C. tetani*, Condrea (53)

soon confirmed the existence of H and O antigens in the species. His smooth strains were complete with both antigens, whereas the rough possessed only the O. The O antigen of any given rough strain, however, was like that of the smooth strains of its group. In other words, overlapping or very close relationship of O antigens between the groups was not confirmed. In fact, Condrea stated clearly that in his opinion such cross-reactions must be due to admixture of types! There the matter rested until Gunnison (109) recently presented a thorough study of 67 strains, representing all nine of the types now known. As a result of the cross-testing of all strains with the antisera for all nine groups, "there was no differentiation among the 67 strains tested." Absorption tests being confirmatory, it was concluded that there is a common O antigen with no type specificity. But in addition to this common O antigen, absorption tests revealed another O antigen, which divides the collection into two subgroups. Types II, IV, V, and IX possess this second O antigen, whereas I, III, VI, VII, and VIII lack it. The complexity of agglutination of *C. tetani* is therefore still considerable, but it has been clarified; and it should now be possible to identify an unknown strain as belonging to the species, *if* it possesses the common O or species-specific factor. *Clostridium tetanomorphum* was tested by Gunnison and, although it gave a group reaction to 1:640 titre, absorption with it did not lower the original O titre of the serum for *C. tetani*, thereby proving that *C. tetanomorphum* has not the species-specific O factor of *C. tetani*. *Clostridium tertium*, *Clostridium putrificum*, and *Clostridium sphenoides* were found to be even further removed in group relation. Among the 67 strains of *C. tetani* tested 11 were atoxic and therefore could not have been recognized as belonging to the species by a toxin-antitoxin test.

The agglutination of Clostridium septicum

Some early studies on the agglutination of *C. septicum* were made to distinguish it from *C. chauvoei* (178, 194, 283), apparently with success; although recently "a close relation" between the two organisms has been indicated by a combination of evi-

dence from their agglutination and complement fixation reactions (349). The complexity of its agglutination first became apparent in the report by Robertson (264) which indicated three subtypes in the species. Later, when flagellar-somatic agglutination was being applied to the species by Felix and Robertson (83), the same sera were reinvestigated, although nearly nine years old. It was found that the original subdivision depended upon the H antigen reactions, in this species as in *C. tetani.* Four groups were finally established by Felix and Robertson. In the somatic sera, they found the cross-reaction of the O antigens of the several groups to indicate close group relationship, but not identity. In 1928 Davesne (71) mentioned six subgroups in direct agglutination and therefore presumably dependent upon H antigens, but Bengtson (19) in 1933 still recognized only "at least four" groups.

The somatic or O antigen of *C. septicum* has an added importance in relation to protective immune sera, and has therefore been investigated by several workers. Robertson and Felix (266) claimed definite, but type-specific, protective value of the O immune serum. Weinberg, Davesne and Haber (348) were unable to confirm its value, and since antitoxic sera were successful, they saw no reason to change therapeutic procedure. Henderson, (139, 140), continuing investigation of the O antigen both for its species relationship and for its possible prophylactic value, confirmed the Robertson-Felix report. He did, however, clarify their statement concerning the close relation of the O antigens of the several groups. There is an identical O antigen in Groups II and IV; and "considerable overlapping," but not identity, in I and III. In conclusion, he suggested that the O antigen relationship be made the primary basis for grouping within the species, and that the H antigen relations be considered secondary. This suggestion has not been widely recognized, but it is, in fact, a good one, as the O antigen is the more stable. Henderson's most recent contribution (141) suggests that in addition to the O antigen, there is a heat-labile antigen, possibly identical with the H antigen, which has importance in antibacterial sera.

The agglutination of Clostridium chauvoei

After the early separation of *C. chauvoei* from *C. septicum*, the agglutination of *C. chauvoei* itself was examined (156, 226, 377, 378, 381). Strong reactions with "no serological differentiation of races" were found to occur, regardless of the ovine or bovine origin of the strains. Nevertheless, controversy as to the identity of so-called "spontaneous Rauschbrand" of sheep *versus* the "wound Rauschbrand" of cattle (209, 225, 226, 370) was not finally settled until a full antigenic analysis of the species by flagellar-somatic agglutination was accomplished. On this basis the H antigen was found by Roberts (260) to be different in the ovine and bovine strains, whereas the O was identical. Henderson (136), repeating the study, agreed upon the uniformity of the O factor; in fact, he claimed exceptional importance for it in view of the subdivision of species by means of the O factor in the case of *C. septicum*. He did not, however, confirm the separate entity of the ovine and bovine H factors. Instead he found an equal distribution of the H antigen in all but two of his strains (both English ovine which had a common H factor different from that of the other strains). A minor component of the H factor is shared by all strains. Later work deals only with the immunizing value of the O antigen in vaccine (137, 261) or in antibacterial serum (138).

The agglutination of Clostridium parabotulinum and Clostridium botulinum

The first investigators to study extensively the agglutination of *C. parabotulinum* (then called *B. botulinus*) were Starin and Dack (305) and Schoenholz and Meyer (281). The latter, examining 111 strains of diverse origins, found that the Type A strains could be divided into three or four subgroups and the Type B strains into at least two subgroups by agglutination and absorption tests. In each toxin type one group was left a heterogeneous collection of ultra-specific and unclassified strains. Only between certain groups was cross-agglutination noticed. Generally, negative results were obtained on cross-tests of the botulinus and the non-toxin-producing anaerobes, such as *C. sporogenes* and *C. bifermentans*.

Using the flagellar-somatic agglutination technique in an extensive re-study of over 160 cultures of the proteolytic *C. parabotulinum*, including the original collection of Schoenholz and Meyer, McClung (186) showed that the subgroups reported by previous workers were based on the reaction of the heat-labile antigens. Without exception, strains of *C. parabotulinum* Types A and B reacted with a serum produced against the heat-treated antigen of any strain regardless of toxin type. Mirror absorption experiments confirmed the identity of the heat-stable somatic antigen. Townsend (322) and Gunnison and Meyer (113) had previously classified various non-toxic strains by their reaction to titre with specific antisera; these strains also reacted with the somatic antisera and could not be distinguished from the toxic cultures. Even more interesting is the possibility of using a somatic antiserum as a specific reagent for the proteolytic *C. parabotulinum*, as revealed by the cross-tests of these organisms and various related species, in particular *C. sporogenes*. Although some cross-reaction is evident prior to absorption, it appears that somatic antiserum for *C. parabotulinum*, preabsorbed by *C. sporogenes*, may give a reaction with the homologous species only. However, the strains of *C. sporogenes* do not all react uniformly; therefore a decision as to the group relationship of this species to *C. parabotulinum* must await further examination. Nevertheless, the great importance of such a serum is evident, and it is hoped that the future will soon produce a fuller study.

The heat-stable antigens of a small number of strains of Types B, C and D of *C. botulinum* were included in McClung's study (186), and their apparent specificity was noted. Prior to this, the agglutination of Types C and D had been examined by several workers (110, 224, 248, 268), and they too had found no cross-reaction with the type sera of *C. parabotulinum*.

The agglutination of Clostridium welchii

It is not yet possible to present the agglutination of *C. welchii* in anything like the state of agreement that has been reached for *C. tetani* and *C. parabotulinum*. There is nothing but confusion at present, and apparently for two reasons. As late as 1929,

Robertson (265) wrote that "there is no evidence that agglutinins have been produced in the bodies of animals injected with *B. welchii*." It is true that the literature contains rather numerous statements that such agglutinins are not, or at least not readily, produced. That they can be formed, however, is shown in some of the early studies. Several recent reports (149, 259, 344, 367, 375) of even comparatively high titres have removed all doubt on this score. However, the second difficulty, namely, that the antisera are conspicuously strain-specific, has not been overcome. We can do no more than quote Wilsdon (367): "While it is possible to prepare agglutinating sera in the case of a number of strains of *B. welchii*, there is little likelihood of formulating a satisfactory classification of the members of that group on the basis of their agglutination reactions." It is only fair to point out, however, that he refers to the entire *C. welchii* group, human and animal. It must also be pointed out that *C. welchii* has not been fully analyzed for antigenic structure by the newer techniques. The organism is non-motile, an exception to most of the group of spore-forming anaerobes. For this reason it has no flagellar antigen; but it does have capsular antigens to be considered. The precipitin and complement fixation reactions, which incidentally have not been adequately tried for the species, should offer possibilities.

The agglutination of some proteolytic species

It is convenient to consider together the agglutination of a group of non-pathogenic proteolytic anaerobes. There is as yet no certainty of a subdivision into types within these species, but there is interrelation of certain species, as recently revealed by agglutination studies.

Clostridium sporogenes is probably poorly defined, in the sense that it is considered a widely distributed species and that non-pathogenic proteolytic forms are often assigned to it without detailed study. Perhaps then confusion is to be expected. The several reports (75, 128, 200, 246, 378, 379) are not in agreement, except upon the fact that not all strains of *C. sporogenes* agglutinate in all antisera. Three groups were recognized by Zeissler and

Rassfeld (379), but Hall and Stark (128) claimed agglutination, if only to low titre, with all strains. Lack of homogeneity is again indicated in the two recent reports upon cross-agglutination of *C. sporogenes* and certain other proteolytic species. McClung (186) noted irregularity of behavior of certain strains of *C. sporogenes* in absorption of the antisera for *C. parabotulinum*, as discussed above. Another example of the interrelation of "certain strains" of *C. sporogenes* with another anaerobic species is reported by Smith (292). Stable rough variants of *C. histolyticum* are said to react, though not to full titre, in an antiserum for *C. sporogenes* (strain 319 only); a reciprocal reaction was also obtained with this strain. Other strains of *C. sporogenes* reacted only to 1:20 or 1:80 dilution of antisera for the rough variants of *C. histolyticum*. These findings were offered in support of a claim (147) that rough variants of *C. histolyticum*, which resemble *C. sporogenes* in colony form and certain biochemical properties, are not chance contaminants but show "genetic relation" to *C. sporogenes*. Smith, however, does not claim direct identity of the species, but merely "a number of antigens common to both *Clostridium sporogenes* and *Clostridium histolyticum*." Further study will be necessary to establish these relations.

Clostridium bifermentans has been studied recently in order to clarify its relation to the so-called *Bacillus centrosporogenes*. Since one of the principal differences claimed was the absence of motility in *C. bifermentans* and its presence in *B. centrosporogenes*, a study of the flagellar and somatic antigens seemed indicated. Proof of the complete identity of the antigens, coupled with the previously known identity of other characters makes it no longer necessary to recognize two species. Because *C. bifermentans* has priority, its name has been retained (47, 192). The merging of another species, *C. sordellii*, with *C. bifermentans* has recently been proposed, partly on the basis of cross-agglutination. Details of this proposal by Stewart (310) have been discussed under the section on the toxin of *C. oedematoides* (syn. *C. sordellii*).

Several other putrefactive species have been studied in recent years by Hall and his associates, and in each case agglutination has aided in establishing the species: *Bacillus paraputrificus*

Bienstock and *Bacillus capitovalis* Snyder and Hall (295, 297); *Bacillus difficilis* Hall and O'Toole, in which subgroups were recognized by Snyder (296); *Clostridium fallax* and *Clostridium carnis* (77); *Bacillus bifermentans* as separate from several others (47); and *Bacillus paraputrificus* Bienstock and *"Bacillus innutritus"* Kleinschmidt (124).

The agglutination of the saccharolytic anaerobes of the butyric group

Butyric anaerobes of the soil are a heterogeneous lot, and no one knows how many species to recognize! Serology has not given a complete answer by any means, but it has, we believe, contributed to the definition of several species (188, 190, 191). One such is *Clostridium acetobutylicum*, the organism of the original Weizmann method of butyl alcohol fermentation. An unusually complete set of cultures was available for the serological study of the species: two representatives of the original Weizmann isolation (one untouched for 17 years) and twenty other cultures isolated by various workers over a period of 20 years. A complete antigenic analysis was made by the H and O technique by both direct agglutination and absorption (190). Unquestionably, every strain belonged to the species, and there was no need to subdivide the group into types on the basis of either flagellar or somatic agglutination. The only variation encountered was quantitative, in the sense of Schütze (282); namely, certain strains were *master strains* with full complement of H and O factors, and others were *substrains* with the same antigens, but with some deficiency of the H factor. Since the latter is the flagellar antigen, it is not surprising that quantitative variation should occur from time to time.

In the group reaction of *C. acetobutylicum* with other butyric anaerobes there is nearest affinity to the retting organism, *Clostridium felsineum* Carbone, and to a new pigmented anaerobe which appeared different and which was later proposed as a new species, *Clostridium roseum* McCoy and McClung (191). The interrelations of the three species were analyzed with the following results (188). A common somatic O factor occurs in the three organisms, but each is distinguished by an H factor, which

is dominant and species-specific. The group reaction in the *C. felsineum: C roseum* and *C. felsineum: C. acetobutylicum* crosses is accounted for entirely by the common O factor. That between *C. acetobutylicum* and *C. roseum* is due to the O factor plus some admixture of H factors, the *C. acetobutylicum* containing a minor fraction of the H which is dominant in *C. roseum*. Thus, serological evidence confirmed in another instance the division of species proposed on morphological and physiological grounds.

Another butyric anaerobe, *Clostridium thermosaccharolyticum* McClung (185), was analyzed by the same technique (187). It also is homogeneous, with variation only in the quantity of H factor in certain strains. The extension of flagellar-somatic agglutination to this species is of particular interest, because, as a thermophile, the organism grows at a temperature which would destroy the H antigen of a mesophilic species. Yet relatively, the H factor of the thermophile is thermolabile and comparable to the same factor in other motile species (189).

THE APPLICATION OF THE PRECIPITIN AND COMPLEMENT FIXATION REACTIONS TO ANAEROBES

The precipitin reaction

The close analogy between the precipitin and agglutinin reactions is well-known. Although much less antigenic analysis has been done by precipitation, there is a good agreement of results obtained thus far by the two methods as applied to the spore-forming anaerobes. Detection of precipitins in the diagnosis of diseases has been attempted (91, 95, 133, 247, 270, 372), and precipitating sera for a number of the species of anaerobes have been successfully prepared (30, 40, 81, 108, 116, 351). Precipitins can often be demonstrated with antisera prepared for general antimicrobial use (302). Weinberg and Barotte (344) have claimed that precipitins and agglutinins act together in a serum to give stronger reactions ("synergism of antibodies") without loss of specificity.

Probably the most extensive work on precipitation for the typing of an anaerobic species has been done with *C. parabotulinum* by Gunnison and Schoenholz (116). A large collection of Type A

and B strains was tested in precipitating antisera produced with washed and heated cells, and good agreement with the grouping by agglutination was found. Later (108), antisera were produced with the bacterial cell extracts obtained by the freezing-thawing technique. Such extracts contained both protein and carbohydrate constituents, but they were not antigenic *in vivo;* they were, however, specifically precipitable by antisera produced against the intact organisms.

Comparatively little is known of the specific carbohydrate constituents of the anaerobes. Jimenez (155) reported simply that such a fraction had been isolated from *C. welchii,* and Meisel (218) reported similarly for a *Bacillus amylobacter.*

The complement fixation reaction

Much the same success can be claimed for typing by complement fixation as by agglutination and precipitation, but the method has been even less extensively used. It has had its most considerable trial with the botulinus organism (115, 306,) and the tetanus organism (83). Highly specific reactions with groupings closely following those revealed by agglutination have been found. Gunnison and Schoenholz (115) pointed out one advantage: namely, that certain so-called inagglutinable strains can be assigned to groups by complement fixation.

Mention might also be made of an incidental use of the complement fixation reaction for detecting the botulinus organism or its toxin in spoiled canned foods (160, 306). It is also possible to determine with a high degree of accuracy the toxin content of a culture (308).

This brief presentation of the precipitation and complement fixation phenomena of the anaerobes is not in proportion to the importance of these reactions. But it cannot be claimed that either of them has contributed much to the typing of anaerobes, except to confirm previously known groupings. Precipitin tests will doubtless contribute significantly, after the groundwork has been laid by studies of the specific soluble substances of these species.

A CRITIQUE OF SEROLOGY AS AN AID IN THE TAXONOMY OF ANAEROBES

Every bacteriologist must be troubled at times by problems of taxonomy. He may not himself be concerned with the technique and terminology of classification, but he would like to have *agreement* and *stability* as quickly as possible. To be told that bacteria are perhaps not classifiable is of no help. To be told that they can be classified only when more is known is of no present help. Nor is this quite true now, for there are groups of bacteria *within* which classification (in the common sense) has already been achieved. It is chiefly when he tries to comprehend the whole system of bacteria that he must conclude that taxonomy has failed. Yet he would probably still hold with Lehmann and Neumann (180) "that it is always necessary to strive after such a system."

The known best way to work toward a general classification is to build up one after another of the systems for special groups; and in that endeavor serological classification has its place. One can hardly expect to take an unknown organism, and knowing nothing of its morphology and physiology, to discover its identity by toxin neutralization. It may perhaps, produce no toxin. But one can, given a spore-forming anaerobe from the pulpy kidney, of a sheep, discover the toxin type. And having done so, one would have classified it intelligibly for others working with the *C. welchii* group. *Agreement* would be reached.

As to the *stability* of serological classification, it is possible to find arguments, both favorable and unfavorable. The major objections are that variation "changes" the antigenic reactions and that serology reveals a needlessly complex subdivision of organisms (which is perhaps another way of saying that antigenic variation has occurred). If the antigenic variation is so random that types are forever elusive, the objection is valid. But experience shows this not to be so. The very fact that a group of botulinus cultures, involving hundreds of isolations, can be sorted into five toxin types is significant. So too is the statement of Powell (251): "Uniformity in the results of agglutina-

tion and agglutinin absorption tests upon groups of single-cell cultures of the diphtheria bacillus, having common origins, indicates considerable stability in the agglutinative reactions of this organism. It has not been possible to split any parent culture on the basis of the agglutinative reaction of pure-line strains derived from it."

We do not imply that serological analyses have solved all problems of classification within groups. Certainly that cannot be claimed for any single method of serology. Agglutination of *C. welchii*, for example, has failed for reasons as yet unknown. We are aware also that there is no convenience in the considerable subdivision of *C. tetani* by its H antigen reactions; but we submit that the discovery of a common and species-specific O factor is useful. That the O factor of *C. parabotulinum* unites a part of the botulinus group is probably also significant. So also is the possible protective power of the "O vaccines" of *C. chauvoei*. So is the sharing of O factors between species in the butyric group. In short, it seems that *somatic* factors are most promising objects for further study. It is apparent also that group reactions require attention. The sharing of minor somatic elements among certain species (or among groups within a species) may show phylogenetic relationships useful to know. And conversely, removal of those group factors by pre-absorption of antisera may yield useful reagents for species analyses. One such case among the anaerobes has been indicated in discussion of *C. parabotulinum* and *C. sporogenes*.

Needless to say, all serological work requires great care. Improvement in purity and standardization of toxins and antitoxins, and greater use of cross-protection experiments for establishing the components of nearly related crude toxins, are the keys to further progress. In agglutination work greater emphasis must be placed on *complete* analyses with mirror absorptions. And finally, with precipitation reactions done upon isolated and if possible chemically defined fractions of protein and polysaccharide, it may be possible to reach that "Substantive Classification," which P. Bruce White has visioned (365).

REFERENCES

(1) 1935 Report of the inter-governmental conference on biological standardisation. League Nations, Health Organ., Quart. Bull., **4**, 631–642.

(2) ABEL, J. J. 1934 On poisons and disease and some experiments with the toxin of the *Bacillus tetani*. Science, n. s. **79**, 63–70; 121–129.

(3) ABEL, J. J., EVANS, E. A., AND HAMPIL, B. 1936 Researches on tetanus. V. Distribution and fate of tetanus toxin in the body. Bull. Johns Hopkins Hosp., **59**, 307–391.

(4) ABEL, J. J., EVANS, E. A., HAMPIL, B., AND LEE, F. C. 1935 Researches on tetanus. II. The toxin of the *Bacillus tetani* is not transported to the central nervous system by any component of the peripheral nerve trunks. Bull. Johns Hopkins Hosp., **56**, 84–114.

(5) ABEL, J. J., AND HAMPIL, B. 1935 Researches on tetanus. IV. Some historical notes on tetanus and commentaries thereon. Bull. Johns Hopkins Hosp., **57**, 343–372.

(6) ABEL, J. J., HAMPIL, B., AND JONAS, A. F. 1935 Researches on tetanus. III. Further experiments to prove that tetanus toxin is not carried in peripheral nerves to the central nervous system. Bull. Johns Hopkins Hosp., **56**, 317–336.

(7) ADEY, C. W. 1935 [Anaerobic infections] Brit. Med. Jour., **2**, 748.

(8) ALLEN, H. R., AND BOSWORTH, T. J. 1924 Immunisation of animals against infection by *Vibrion septique* and *Bacillus chauvoei*. Vet. Jour., **80**, 344–362.

(9) AOKI, M. 1935 Agglutinatorische Untersuchung von Aktinomyzeten. Zeitschr. Immunitätsf., **86**, 518–524.

(10) AOKI, M. 1936 Beziehung der agglutinatorischen Einteilung der Aktinomyzeten zu der nach der Komplementbindungsreaktion. Zeitschr. Immunitätsf., **87**, 200–201.

(11) AOKI, M. 1936 Ueber die agglutinatorische Bedeutung von Arthrosporen bei Aktinomyzeten. Zeitschr. Immunitätsf., **88**, 60–62.

(12) AOKI, M. 1936 Weitere agglutinatorische Untersuchung der Aktinomyzeten. Zeitschr. Immunitätsf., **87**, 196–199.

(13) BACHMANN, W., UND GREGOR, H. 1936 Kulturelle und immunobiologische Differenzierung von Stämmen der Gruppe "Fusobakterium." Zeitschr. Immunitätsf., **87**, 238–251.

(14) BAUER, J. H., AND MEYER, K. F. 1926 Human intestinal carriers of tetanus spores in California. Jour. Infect. Diseases, **38**, 295–305.

(15) BEHRING, —, UND KITASATO, —. 1890 Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. Deut. med. Wchnschr., **16**, 1113–1114.

(16) BENGTSON, I. A. 1922 Preliminary note on a toxin-producing anaerobe isolated from the larvae of *Lucilia caesar*. U. S. Pub. Health Rpts., **37**, 164–170.

(17) BENGTSON, I. A. 1922 Separation of toxic and nontoxic cells from cultures of an anaerobe isolated from larvae of the green fly. U. S. Pub. Health Rpts., **37**, 2252–2256.

(18) BENGTSON, I. A. 1924 Studies on organisms concerned as causative factors in botulism. U. S. Pub. Health Serv., Hyg. Lab. Bull. No. **136**, 1–101.

(19) BENGTSON, I. A. 1933 The potency testing of *Vibrion septique* antitoxin. Jour. Bact., **25**, 84–85.

(20) BENGTSON, I. A. 1934 Studies on the standardization of *Vibrion septique* antitoxin. U. S. Pub. Health Rpts., **49**, 251–262.

(21) BENGTSON, I. A. 1934 The standardization of gas gangrene (*perfringens*) antitoxin. U. S. Pub. Health Rpts., **49**, 525–529.

(22) BENGTSON, I. A. 1934 The official United States and International unit for standardizing gas gangrene antitoxin (*Vibrion septique*). U. S. Pub. Health Rpts., **49**, 1557–1569.

(23) BENGTSON, I. A. 1936 The official United States and International unit for standardizing gas gangrene antitoxin (*oedematiens*). U. S. Pub. Health Rpts., **51**, 266–275.

(24) BENGTSON, I. A., AND STEWART, S. E. 1936 The official United States and International unit for standardizing gas gangrene antitoxin (*histolyticus*). U. S. Pub. Health Rpts., **51**, 1263–1272.

(25) BENNETTS, H. W. 1932 Infectious entero-toxaemia (the so-called braxy-like disease) of sheep in Western Australia. Austral. Inst. Sci. Ind., Bull. No. **57**, 1–72.

(26) BENNETTS, H. W. 1932 "Pulpy kidney"—a *post-mortem* change in experimental infectious entero-toxaemia. Austral. Inst. Sci. Ind., Pamphlet No. **35**, 26–29.

(27) BENNETTS, H. W. 1933 The active immunization of sheep against infectious entero-toxaemia (braxy-like disease) by means of *B. ovitoxicus* anaculture. Jour. Council Sci. Ind. Research, **6**, 92–98.

(28) BENNETTS, H. W. 1936 The control of entero-toxaemia of sheep. Austral. Vet. Jour., **12**, 196–202.

(29) BERGEY, D. H. 1934 *Manual of Determinative Bacteriology*. Williams & Wilkins, Baltimore. 4th Ed., 664 pp.

(30) BOHNOFF, —. 1917 Ueber Gasphlegmone. Münch. med. Wchnschr., **64**, 762–763.

(31) BORTHWICK, G. R. 1935 Ueber die antigenen Eigenschaften der Gifte der zur Gruppe des Welch-Fraenkelschen Gasödembazillus gehörenden Mikroorganismen, nebst Bemerkungen über die experimentelle Erzeugung enterogener Anaerobeninfektionen bei Schafen. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **134**, 289–302.

(32) BORTHWICK, G. R. 1936 Pathogenic anaerobic bacteria. Intern. Soc. Microbiol., 2nd Cong., 180.

(33) BORTHWICK, G. R. 1936 Untersuchungen über die Wertbemessung des antitoxischen Oedematiensserums. Zeitschr. Immunitätsf., **87**, 389–399.

(34) BORTHWICK, G. R. 1937 Observations on *B. welchii* Type D: Its occurrence in normal animals and the variation in antigenic character of its toxin. Brit. Jour. Expt. Path., **18**, 475–481.

(35) BORTHWICK, G. R., AND GRAY, J. D. A. 1937 The type of *Cl. welchii* in human faeces, with special reference to pernicious anaemia. Brit. Jour. Expt. Path., **18**, 119–125.

(36) BOSWORTH, T. J. 1936 The rôle of *Clostridium welchii* and other anaerobes in disease of sheep. Intern. Soc. Microbiol., 2nd Cong., 179–180.

(37) BOSWORTH, T. J., AND GLOVER, R. E. 1934–35 A differential character of *Clostridium welchii* Type D. Proc. Roy. Soc. Med., **28**, 1004–1006.

(38) BOSWORTH, T. J., AND GLOVER, R. E. 1934–35 The effect on *Cl. welchii* toxins of a substance present in the normal intestine. Univ. of Cambridge, Inst. of Anim. Path., 4th Rpt. of the Director, 79–93.

(39) BOSWORTH, T. J., AND JORDAN, L. 1929 *Bacillus oedematiens* infection in cattle. Vet. Jour., **85**, 393–399.

(40) BRONFENBRENNER, J. J., AND REICHERT, P. 1926 The nature of the toxin-antitoxin flocculation phenomenon. Jour. Expt. Med., **44**, 553–565.

(41) BROWN, J. H. 1937 The study of obligately anaerobic bacteria. *Manual of Methods for Pure Culture Study of Bacteria*, Leaflet III, 3rd Ed., 13.

(42) BULL, C. G. 1917 The prophylactic and therapeutic properties of the antitoxin for *Bacillus welchii*. Jour. Expt. Med., **26**, 603–611.

(43) BULL, C. G., AND PRITCHETT, I. W. 1917 Identity of the toxins of different strains of *Bacillus welchii* and factors influencing their production *in vitro*. Jour. Expt. Med., **26**, 867–883.

(44) BULL, C. G., AND PRITCHETT, I. W. 1917 Toxin and antitoxin of and protective inoculation against *Bacillus welchii*. Jour. Expt. Med., **26**, 119–138.

(45) BURKE, G. S. 1919 Notes on *Bacillus botulinus*. Jour. Bact., **4**, 555–565.

(46) CELAREK, J., ET STETKIEWICZ, S. 1936 Contribution a l'étude des hémotoxines de la gangrène gazeuse. Compt. rend. soc. biol. (Paris), **122**, 143–144.

(47) CLARK, F. E., AND HALL, I. C. 1937 A comparative study of *Bacillus bifermentans* (Tissier and Martelly), *Bacillus centrosporogenes* (Hall) and certain closely related proteolytic anaerobes. Jour. Bact., **33**, 23–24.

(48) COLEF, I. 1935 Dissociation du *Bacillus oedematiens*. Compt. rend. soc. biol. (Paris), **119**, 684–685.

(49) COLEMAN, G.-E. 1915 L'acide butyrique et la sclérose. Ann. inst. Pasteur, **29**, 139–156.

(50) COLEMAN, G. E., AND GUNNISON, J. B. 1928 Serologic types of *Clostridium tetani*. Jour. Infect. Diseases, **43**, 184–188.

(51) COLEMAN, G. E., AND MEYER, K. F. 1926 Study of tetanus agglutinins and antitoxin in human serums. Jour. Infect. Diseases, **39**, 332–336.

(52) Committee upon anaerobic bacteria and infections. 1919 Anaerobic infections of wounds and the bacteriological and serological problems arising therefrom. [Gt. Brit.] Med. Research Council, Spec. Rpt. Ser. No. **39**, 1–182.

(53) CONDREA, P. 1930 Recherches sur les propriétés antigéniques des races "smooth" et "rough" du bacille tétanique. Arch. roumaines path. exptl. microbiol., **3**, 365–374.

(54) CONNELL, H. C. 1935 The study and treatment of cancer by proteolytic enzymes: a preliminary report. Can. Med. Assoc. Jour., n. s. **33**, 364–370.

(55) CONRADI, H., UND BIELING, R. 1916 Zur Aetiologie und Pathogenese des Gasbrands. II. Münch. med. Wchnschr., **63**, 1023–1025; 1068–1070.

(56) COURMONT, J. 1898 De l'agglutination du bacille de Nicolaïer par le sérum d'animaux normaux, tétaniques ou immunisés. Compt. rend. soc. biol. (Paris), **50**, 1107–1109.

(57) COURMONT, J. 1899 Deuxième note sur l'agglutination du bacille de Nicolaïer. Compt. rend. soc. biol. (Paris), **51**, 163–164.

(58) CRUICKSHANK, J. C. 1935–36 Modern methods in agglutination. Proc. Roy. Soc. Med., **29**, 841–854.

(59) DACK, G. M., DRAGSTEDT, L. R., AND HEINZ, T. E. 1936 *Bacterium necrophorum* in chronic ulcerative colitis. Jour. Am. Med. Assoc., **106**, 7–10.

(60) DACK, G. M., DRAGSTEDT, L. R., AND HEINZ, T. E. 1937 Further studies on *Bacterium necrophorum* isolated from cases of chronic ulcerative colitis. Jour. Infect. Diseases, **60**, 335–355.

(61) DACK, G. M., HEINZ, T. E., AND DRAGSTEDT, L. R. 1935 Ulcerative colitis. Study of bacteria in the isolated colons of three patients by cultures and by inoculation of monkeys. Arch. Surg., **31**, 225–240.

(62) DALLING, T. 1928 Continuation of paper on lamb dysentery, published in Handbook of Animal Congress, National Veterinary Medical Association of Great Britain and Ireland, pp. 55–72. Vet. Rec., n. s. **8**, 841–849.

(63) DALLING, T. 1934 Note. Vet. Jour., **90**, 337.

(64) DALLING, T. 1936 Anaerobic infections of sheep. Vet. Rec., n. s. **16**, 1477–1480.

(65) DALLING, T. 1936 Opener: The prophylaxis and serum treatment of human and animal diseases caused by anaerobic bacteria. Intern. Soc. Microbiol., 2nd Cong., 491–493.

(66) DALLING, —., HUGHES, T., WOODRUFF, H. A., AND GAIGER, S. H. 1924 Anaerobic infections of animals. Discussion on Professor Gaiger's paper. Vet. Rec., n. s. **4**, 1043–1051.

(67) DALLING, T., McEWEN, A. D., ROBERTSON, M., WILLIAMS, B. W., WRIGHT, D., NEAME, H., AND IVENS-KNOWLES, —. 1931–32 Discussion on *B. welchii* infections in animals and man. Proc. Roy. Soc. Med., **25**, 807–817.

(68) DALLING, T., MASON, J. H., AND GORDON, W. S. 1927 Prophylaxis of lamb dysentery. Jour. Comp. Path. Therap., **40**, 217–223.

(69) DALLING, T., MASON, J. H., AND GORDON, W. S. 1928 Lamb dysentery prophylaxis in 1928. Vet. Jour., **84**, 640–648.

(70) DALYELL, E. J. 1917 A case of gas gangrene associated with *B. oedematiens*. Brit. Med. Jour., **1**, 361–362.

(71) DAVESNE, J. 1928 Races sérologiques du *Vibrion septique*. Compt. rend. soc. biol. (Paris), **99**, 763–764.

(72) DEBONÉRA, G. 1934 Contribution à l'étude de la dysentérie des agneaux. Rec. méd. vét., **110**, 524–534.

(73) DEBONÉRA, G., ET ANANIADÈS, B. 1935 Les gastro-entéro-toxémies infectieuses (bradsot du mouton), Rev. gén. méd. vét., **44**, 385–408.

(74) DOERR, R. 1937 Wanderungen von Toxin und Virus in peripheren Nerven. Schweiz. med. Wchnschr., **67**, 329–334.

(75) DOUGLAS, S. R., FLEMING, A., AND COLEBROOK, L. 1920 Studies in wound infections. [Gt. Brit.] Med. Research Council, Spec. Rpt. Ser. No. **57**, 1–159.

(76) DUBOVSKY, B. J., AND MEYER, K. F. 1922 An experimental study of the methods available for the enrichment, demonstration and isolation of *B. botulinus* in specimens of soil and its products, in suspected food, in clinical and in necropsy material. I. Jour. Infect. Diseases, **31**, 501–540.

(77) DUFFETT, N. D. 1935 The differentiation of *Bacillus fallax* (Weinberg and Séguin) from *Bacillus carnis* (Klein). Jour. Bact., **29**, 573–581.

(78) EATON, M. D. 1936-37 Purification of tetanic toxin. Proc. Soc. Expt. Biol. Med., **35**, 16–19.

(79) EGGERTH, A. H. 1935 The gram-positive non-spore-bearing anaerobic bacilli of human feces. Jour. Bact., **30**, 277–299.

(80) EGGERTH, A. H., AND GAGNON, B. H. 1933 The *Bacteroides* of human feces. Jour. Bact., **25**, 389–413.

(81) FALCHETTI, E. 1935 De la production comparée des précipitines au moyen d'injection de sérum seul ou enrobé dans la lanoline. Compt. rend. soc. biol. (Paris), **118**, 1041–1043.

(82) FELIX, A., AND GARDNER, A. D. 1937 The serum diagnosis of enteric fever. Report and recommendations. League Nations, Health Organ., Bull. **6**, 223–235.

(83) FELIX, A., AND ROBERTSON, M. 1928 Serological studies in the group of the spore-bearing anaërobes. I. The qualitative analysis of the bacterial antigens of *B. oedematis maligni* (*Vibrion septique*) and *B. tetani*. Brit. Jour. Expt. Path., **9**, 6–18.

(84) FILDES, P. 1925 Tetanus. I. Isolation, morphology and cultural reactions of *B. tetani*. Brit. Jour. Expt. Path., **6**, 62–70.

(85) FILDES, P. 1925 Tetanus. II. Agglutination and toxicity of *B. tetani*. Brit. Jour. Expt. Path., **6**, 91–98.

(86) FILDES, P. 1927 Tetanus. III. Non-toxic variants of *B. tetani*. Brit. Jour. Expt. Path., **8**, 219–226.

(87) FLEMING, W. L. 1927 Studies on the oxidation and reduction of immunological substances. VII. The differentiation of tetanolysin and tetanospasmin. Jour. Expt. Med., **46**, 279–290.

(88) FORD, W. W., AND WILLIAMS, G. H. 1919 Observations on the production of an antihaemotoxin for the haemotoxin of *Bacterium welchii* (*Bacillus aerogenes capsulatus*). Jour. Immunol., **4**, 385–392.

(89) FORSYTH, W. L., AND ZANATY, A. F. I. 1930 The isolation of atoxic strains of *Bacillus tetani* in Egypt. Indian Med. Gaz., **65**, 500–502.

(90) FRIEDEMANN, U., AND ELKELES, A. 1934 The blood-brain barrier in infectious diseases. Its permeability to toxins in relation to their electrical charges. Lancet (London), **1**, 719–724; 775–778.

(91) GAEHTGENS, W. 1917–18 Vergleichende Untersuchungen über die Erreger des Gasbrandes und des malignen Oedems. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **80**, 166–187.

(92) GAIGER, S. H., AND DALLING, T. 1923 Bacillary dysentery in lambs. A note on some recent research into the etiology and source of infection. Jour. Comp. Path. Therap., **36**, 120–125.

(93) GAUTIER, R. 1935 The Health Organization and biological standardization. 2. Tetanus antitoxin. League Nations, Health Organ., Quart. Bull., **4**, 506–508.

(94) GAUTIER, R. 1935 The Health Organization and biological standardization. 4. Gas-gangrene antitoxins. League Nations, Health Organ., Quart. Bull., **4**, 510–512.

(95) GERLACH, F. 1921 Über die Präzipitationsmethode bei Rauschbrand. Zeitschr. Infektionskrank. u. Hyg. Haustiere, **22**, 299–314.

(96) GILBERT, S. J. 1934 Pulpy kidney disease (entero-toxaemia) of sheep in Palestine. Jour. Comp. Path. Therap., **47**, 255–258.

(97) GILL, D. A. 1933 Report on pulpy kidney disease of lambs: an acute entero-toxaemia of bacterial origin. Vet. Jour., **89**, 399–407.

(98) GILL, D. A. 1934 Some aspects of entero-toxaemia in New Zealand. Austral. Vet. Jour., **10**, 212–216.

(99) GLENNY, A. T. 1936 Some anomalies in the titration of antigens and antibodies. Intern. Soc. Microbiol., 2nd Cong., 383.

(100) GLENNY, A. T., BARR, M., LLEWELLYN-JONES, M., DALLING, T., AND ROSS, H. E. 1933 Multiple toxins produced by some organisms of the *Cl. welchii* group. Jour. Path. Bact., **37**, 53–74.

(101) GLOTOWA, E. W., UND DANKEROWITZ, A. K. 1934–35 Zur Standardisierung des Antibotulinusserums. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **133**, 155–158.

(102) GLOTOWA, E. W., UND OSTROWSKAJA, O. A. 1935 Zur Avidität und Wirksamkeit der Tetanusseren. Zeitschr. Immunitätsf., **86**, 146–155.

(103) GLOTOWA, E. W., OSTROWSKAJA, O. A., UND SSILANOWA, I. W. 1934–35 Ueber die Wertbestimmung der Gasödemseren. II. Mitt. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **133**, 415–419.

(104) GLOTOWA, E. W., OSTROWSKAJA, O. A., SSILANOWA, I. W., UND MILASCHEWSKAJA, W. G. 1934 Ueber die Wertbestimmung der Gasödemseren. I. Mitt. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **132**, 57–65.

(105) GORDON, W. S. 1934 Entero-toxaemia in herbivorous animals—its possible bearing on grass sickness in horses. Vet. Rec., n. s. **14**, 1016–1036.

(106) GRAHAM, R., AND BOUGHTON, I. B. 1923 *Clostridium botulinum* type C. A pathogenic anaerobe associated with a limber-neck disease in chickens and ducks. Ill. Agr. Exp. Sta., Bull. No. **246**, 1–34.

(107) GRASSBERGER, R., UND SCHATTENFROH, A. 1902 Ueber den Bazillus des malignen Oedems (*Vibrion septique*). Münch. med. Wchnschr., **49**, 1570–1571.

(108) GUNNISON, J. B. 1934 Studies on the antigenic substances of *Clostridium parabotulinum*. II. Jour. Immunol., **26**, 17–24.

(109) GUNNISON, J. B. 1937 Agglutination reactions of the heat stable antigens of *Clostridium tetani*. Jour. Immunol., **32**, 63–74.

(110) GUNNISON, J. B., AND COLEMAN, G. E. 1932 *Clostridium botulinum*, type C, associated with western duck disease. Jour. Infect. Diseases, **51**, 542–551.

(111) GUNNISON, J. B., CUMMINGS, J. R., AND MEYER, K. F. 1936–37 *Clostridium Botulinum* Type E. Proc. Soc. Expt. Biol. Med., **35**, 278–280.

(112) GUNNISON, J. B., AND MEYER, K. F. 1929 Cultural study of an international collection of *Clostridium botulinum* and *parabotulinum*. XXXVIII. Jour. Infect. Diseases, **45**, 119–134.

(113) GUNNISON, J. B., AND MEYER, K. F. 1929 The occurrence of nontoxic strains of *Cl. parabotulinum*. XXXIV. Jour. Infect. Diseases, **45**, 79–86.

(114) GUNNISON, J. B., AND MEYER, K. F. 1930 Susceptibility of monkeys, goats and small animals to oral administration of botulinum toxin, Types B, C and D. Jour. Infect. Diseases, **46**, 335–340.

(115) GUNNISON, J. B., AND SCHOENHOLZ, P. 1927 Studies on the serologic classification of *B. botulinus*. III. The complement fixation reaction. Jour. Immunol., **13**, 79–92.

(116) GUNNISON, J. B., AND SCHOENHOLZ, P. 1927 Studies on the serologic classification of *B. botulinus*. IV. The precipitin reaction. Jour. Immunol., **13**, 237–241.

(117) GYE, W. E. 1935 Treatment of cancer by proteolytic enzymes. Brit. Med. Jour., **2**, 760.

(118) HAHN, M., UND SKRAMLIK, E. v. 1920 Versuche mit Antigenen und Antikörpern an der überlebenden, künstlich durchströmten Leber. II. Versuche mit Tetanustoxin. Biochem. Zeitschr., **112**, 151–163.

(119) HALL, I. C. 1922 Differentiation and identification of the sporulating anaerobes. Jour. Infect. Diseases, **30**, 445–504.

(120) HALL, I. C. 1929 The occurrence of *Bacillus sordellii* in icterohemoglobinuria of cattle in Nevada. Jour. Infect. Diseases, **45**, 156–162.

(121) HALL, I. C. 1932 The study of obligately anaerobic bacteria. *Manual of Methods for Pure Culture Study of Bacteria*, Leaflet III, 2nd Ed., **14**.

(122) HALL, I. C. 1936 The present status of certain recently discovered anaerobic bacilli. Intern. Soc. Microbiol., 2nd Cong., 170–172.

(123) HALL, I. C., AND PETERSON, E. 1922–23 A note on the mechanism of the peculiar lesions produced by *Bacillus histolyticus*. Proc. Soc. Expt. Biol. Med., **20**, 504–505.

(124) HALL, I. C., AND RIDGWAY, D. 1937 The identity of "*Bacillus innutritus*" (Kleinschmidt) and *Bacillus paraputrificus* (Bienstock). Jour. Bact., **34**, 631–638.

(125) HALL, I. C., RYMER, M. R., AND JUNGHERR, E. 1929 Comparative study of *Bacillus sordellii* (Hall and Scott) and *Clostridium oedematoides* (Meleney, Humphreys and Carp). Jour. Infect. Diseases, **45**, 42–60.

(126) HALL, I. C., AND SCOTT, A. L. 1931 The serologic agglutination of *Bacillus sordellii* and *Clostridium oedematoides*. Jour. Bact., **22**, 375–385.

(127) HALL, I. C., AND SCOTT, J. P. 1927 *Bacillus sordellii*, a cause of malignant edema in man. Jour. Infect. Diseases, **41**, 329–335.

(128) HALL, I. C., AND STARK, N. 1923 Serologic agglutination of *Bacillus sporogenes*. Jour. Infect. Diseases, **33**, 240–247.

(129) HARTLEY, P., AND WHITE, P. B. 1935 A proposed international standard for gas gangrene antitoxin (*Vibrion septique*). League Nations, Health Organ., Quart. Bull., [4] Spec. No., 13–14.

(130) HASLAM, T. P., AND LUMB, J. W. 1919 Blackleg toxin. Jour. Infect. Diseases, **24**, 362–365.

(131) HAUDUROY, P., EHRINGER, G., URBAIN, A., GUILLOT, G., ET MAGROU, J. 1937 Dictionnaire des bactéries pathogènes pour l'homme, les animaux et les plantes. Masson et Cie., Paris, 597 pp.

(132) HAZEN, E. L. 1937 A strain of *B. botulinus* not classified as Type A, B or C. Jour. Infect. Diseases, **60**, 260–264.

(133) HECHT, V. 1912–13 Die Präzipitindiagnose des Rauschbrands, mit einem Beitrag zur Frage der Thermoresistenz der Präzipitinogene. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **67**, 371–381.

(134) HEIDELBERGER, M. 1932, 1933, 1935 Immunochemistry. Ann. Rev. Biochem., **1**, 655–674; **2**, 503–520; **4**, 569–592.

(135) HELLER, H. H. 1920 Etiology of acute gangrenous infections of animals: a discussion of blackleg, braxy, malignant edema and whale septicemia. Jour. Infect. Diseases, **27**, 385–451.

(136) HENDERSON, D. W. 1932 Studies on *Clostridium chauvoei*. I. The analysis of the "H" and "O" antigens of *C. chauvoei*. Brit. Jour. Expt. Path., **13**, 412–420.

(137) HENDERSON, D. W. 1932 Studies on *Clostridium chauvoei*. II. Active immunization with pure "O" antigen. Brit. Jour. Expt. Path., **13**, 421–427.

(138) HENDERSON, D. W. 1933 Studies on *Clostridium chauvoei*. III. Passive protection with pure "O" sera. Brit. Jour. Expt. Path., **14**, 108–112.

(139) HENDERSON, D. W. 1934 Experiments with the "O" antigen of *Clostridium oedematis maligni* (*Vibrion septique*). Brit. Jour. Expt. Path., **15**, 166–175.

(140) HENDERSON, D. W. 1935 The prophylaxis of experimental *V. septique* infection and the practical application of antibacterial methods. Brit. Jour. Expt. Path., **16**, 393–405.

(141) HENDERSON, D. W. 1937 A serological analysis of the protective substances in specific antibacterial sera which control experimental infection with *Cl. oedematis maligni* (*Vibrion septique*). Brit. Jour. Expt. Path., **18**, 224–238.

(142) HENRY, H. 1922 On *B. welchii* haemotoxin and its neutralisation with antitoxin. Jour. Path. Bact., **25**, 1–18.

(143) HENRY, H. 1923 On the composition of *B. welchii* toxin. Jour. Path. Bact., **26**, 497–506.

(144) HENRY, H., AND LACEY, M. 1919–20 On the anaerobes responsible for gas gangrene in man. Jour. Path. Bact., **23**, 281–288.

(145) HENTHORNE, J. C., THOMPSON, L., AND BEAVER, D. C. 1936 Gram-negative bacilli of the genus *Bacteroides*. Jour. Bact., **31**, 255–274.

(146) HOLT, R. L. 1933–34 The pathology of acute strangulation of the intestine. Brit. Jour. Surg., **21**, 582–603.

(147) HOOGERHEIDE, J. C. 1937 Variability in morphological and biochemical properties of *Clostridium histolyticum* (Weinberg and Seguin). Jour. Bact., **34**, 387–408.

(148) HOSOYA, S., TAKADA, M., AND TERAO, S. 1931 On the immunological value of anatoxin derived from purified tetanus toxin. Japan. Jour. Expt. Med., **9**, 33–38.

(149) HOWARD, A. 1928 Races serologiques du *B. perfringens*. Ann. inst. Pasteur, **42**, 1403–1419.
(150) HUMPHREYS, F., AND MELENEY, F. L. 1927–28 The identity of *C. oedematoides* and *B. sordellii*. Proc. Soc. Expt. Biol. Med., **25**, 611–614.
(151) IVANIČ, ST. 1922–23 Über die Erreger des Rauschbrandes der Rinder. Zeitschr. Hyg. Infektionskrank., **97**, 330–345.
(152) JENSEN, C. 1936 Memorandum concerning the titration of tetanus antitoxin. League Nations, Health Organ., Quart. Bull., [5] Spec. No., 702–707.
(153) JENSEN, C. 1936 A proposed international standard for gas-gangrene antitoxin (*histolyticus*). League Nations, Health Organ., Quart. Bull., [5] Spec. No., 659–689.
(154) JERAMEC, C. 1936 Toxine et antitoxine botulique. Rév. Immunol., **2**, 209–220.
(155) JIMENEZ, M.-E. 1931 Sur l'existence d'une substance spécifique hydrocarbonée dans le *Bacillus perfringens*. Compt. rend. soc. biol. (Paris), **106**, 140–141.
(156) JUNGHERR, E. 1928 A comparison of *B. chauvoei* strains from cattle and sheep. Jour. Infect. Diseases, **42**, 84–92.
(157) KADISCH, E. 1923 Das Hämotoxin des Fränkelschen Gasbrandbazillus. Biochem. Zeitschr., **142**, 29–35.
(158) KARUBE, H. 1934 Studien über Toxin von *Vibrion septique*. Japan. Jour. Expt. Med., **12**, 151–168.
(159) KELSER, R. A. 1918 Improved methods of immunization against symptomatic anthrax (blackleg). U. S. Jour. Agr. Research, **14**, 253–262.
(160) KELSER, R. A. 1923 The identification of *Bacillus botulinus* and its toxin in culture and in canned foodstuffs by serological methods. Am. Jour. Pub. Health, **13**, 366–376.
(161) KENDALL, A. I. 1913 Observations on the etiology of severe summer diarrheas of bacterial causation. Boston Med. Surg. Jour., **169**, 754–756.
(162) KENDALL, A. I., AND GEBAUER, E. 1930 The production of histamine by certain strains of the gas bacillus. Jour. Infect. Diseases, **47**, 261–266.
(163) KERRIN, J. C. 1929 The distribution of *B. tetani* in the intestines of animals. Brit. Jour. Expt. Path., **10**, 370–373.
(164) KERRIN, J. C. 1930 Studies on the haemolysin produced by atoxic strains of *B. tetani*. Brit. Jour. Expt. Path., **11**, 153–157.
(165) KERRIN, J. C. 1934 The haemolytic and toxic activities of filtrates of *Clostridium chauvoei*. Jour. Path. Bact., **38**, 219–229.
(166) KITASATO, S. 1889 Ueber den Tetanusbacillus. Zeitschr. Hyg. Infektionskrank., **7**, 225–234.
(167) KLEIN, E. 1894 Ueber nicht virulenten Rauschbrand. Zentr. Bakt. Parasitenk., **16**, 950–952.
(168) KLOSE, F. 1917 Toxin- und Antitoxinversuche mit einem zur Gruppe der Gasödembazillen gehörenden Anaëroben. Münch. med. Wchnschr., **64**, 1541–1542.
(169) KLOSE, F. 1919 Zur Frage der Toxinbildung von Gas-Oedem-Bazillen. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **83**, 305–314.

(170) KOJIMA, K. 1923 Ueber Aktivierung der Bildung giftiger Substanzen von *Bacillus emphysematos* Fränkel durch einige Katalysatoren. Zeitschr. Immunitätsf., **37**, 203–210.

(171) KOJIMA, K. 1923 Über einen neuen Toxinbildner aus der Rauschbrandgruppe. Zeitschr. Hyg. Infektionskrank., **99**, 86–93.

(172) KRANEVELD, F. C. 1930 Bijdrage tot de kennis van de *osteomyelitis bacillosa bubalorum*. Beitrag zur kenntnis der bazillären knochenmarksentzündung der büffel. Nederland. Indische Bl. Diergeneesk., **42**, 564–588.

(173) KRANEVELD, F. C. 1934 Anaërobe Infektionen bei den Haustieren in Niederländisch-Ostindien, besonders über eine specifische Osteomyelitis beim Büffel. Twelfth Intern. Vet. Cong., **2**, 187–200.

(174) LANDSTEINER, K. 1936 *The specificity of serological reactions*. Thomas, Springfield, 178 pp.

(175) LANDSTEINER, K., AND CHASE, M. W. 1937 Immunochemistry. Ann. Rev. Biochem., **6**, 621–643.

(176) LAVERGNE, —. DE 1920 The diagnosis of bacteriological types of gas gangrene by means of specific serums. Lancet (London), **2**, 607–608.

(177) LAVERGNE, V. DE 1918 Gangrène gazeuse. Présence fréquente du *Vibrion septique* et du *Bacillus bellonensis*. Compt. rend. soc. biol. (Paris), **81**, 705–706.

(178) LECLAINCHE, E., ET VALLÉE, H. 1900 Étude comparée du *Vibrion septique* et de la bactérie du charbon symptomatique. Ann. inst. Pasteur, **14**, 590–596.

(179) LECLAINCHE, E., ET VALLÉE, H. 1900 Recherches expérimentales sur le charbon symptomatique. Compt. rend. soc. biol. (Paris), **52**, 139–140.

(180) LEHMANN, K. B., NEUMANN, R. O., AND BREED, R. S. 1931 *Bacteriology, especially determinative bacteriology*. Vol. II, p. 185.

(181) LEMÉTAYER, E. 1936 Hémolysine de la toxine tétanique et antihémolysine. Compt. rend. soc. biol. (Paris), **123**, 742–745.

(182) LEUCHS, J. 1910 Beiträge zur Kenntnis des Toxins and Antitoxins des *Bacillus botulinus*. Zeitschr. Hyg. Infektionskrank., **65**, 55–84.

(183) LIVESAY, H. R. 1933 Dissociation of *Clostridium welchii*. Jour. Infect. Diseases, **53**, 125–137.

(184) McCLEAN, D. 1936 A factor in culture filtrates of certain pathogenic bacteria which increases the permeability of the tissues. Jour. Path. Bact., **42**, 477–512.

(185) McCLUNG, L. S. 1935 Studies on anaerobic bacteria. IV. Taxonomy of cultures of a thermophilic species causing "swells" of canned foods. Jour. Bact., **29**, 189–202.

(186) 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. Jour. Infect. Diseases, **60**, 122–128.

(187) McCLUNG, L. S., AND McCOY, E. 1934–35 Studies on anaerobic bacteria. VIII. The agglutination reactions of *Clostridium thermosaccharolyticum*. Zentr. Bakt. Parasitenk., 2 Abt., **91**, 228–231.

(188) McCLUNG, L. S., AND McCOY, E. 1935 Studies on anaerobic bacteria. VII. The serological relations of *Clostridium acetobutylicum, Cl. felsineum* and *Cl. roseum*. Arch. Mikrobiol., **6**, 239–249.

(189) McCOY, E. 1937 Studies on anaerobic bacteria. XI. On the properties of the H agglutinogens of a mesophilic and a thermophilic species. Jour. Bact., **34**, 321–341.

(190) McCOY, E., AND McCLUNG, L. S. 1935 Studies on anaerobic bacteria. V. The serological agglutination of *Clostridium acetobutylicum* and related species. Jour. Infect. Diseases, **56**, 333–346.

(191) McCOY, E., AND McCLUNG, L. S. 1935 Studies on anaerobic bacteria. VI. The nature and systematic position of a new chromogenic *Clostridium*. Arch. Mikrobiol., **6**, 230–238.

(192) McCOY, E., AND McCLUNG, L. S. 1936 Studies on anaerobic bacteria. IX. Antigenic relations of *Clostridium bifermentans* and *Clostridium centrosporogenes*. Jour. Bact., **31**, 557–568.

(193) McCOY, G. W. 1936 International standardization of biological products by the League of Nations. Am. Jour. Pub. Health, **26**, 1215–1218.

(194) M'EWEN, A. D. 1926 Quarter-evil and braxy: studies regarding immunity. Jour. Comp. Path. Therap., **39**, 253–283.

(195) McEWEN, A. D. 1929–30 *B. paludis:* a new species of pathogenic anaerobic bacterium. Jour. Comp. Path. Therap., **43**, 1–21.

(196) McEWEN, A. D. 1932 Anaerobic infections of sheep as seen in the Romney Marsh. Vet. Rec., n. s. **12**, 1069–1082.

(197) McEWEN, A. D. 1933 "Struck." Enteritis and peritonitis of sheep caused by a bacterial toxin derived from the alimentary canal. II. Jour. Comp. Path. Therap., **46**, 108–125.

(197a) McEWEN, A. D. 1936 The *Bacillus welchii* toxaemias of sheep. Intern. Soc. Microbiol., 2nd Cong., 494–495.

(198) McEWEN, A. D., AND ROBERTS, R. S. 1931 "Struck." Enteritis and peritonitis of sheep caused by a bacterial toxin derived from the alimentary canal. Jour. Comp. Path. Therap., **44**, 26–49.

(199) McGAUGHEY, C. A. 1933 The separation from *Clostridium welchii* of variants which differ in toxicity and antigenic structure. Jour. Path. Bact., **36**, 263–272.

(200) McINTOSH, J., AND FILDES, P. 1917 The classification and study of the anaerobic bacteria of war wounds. [Gt. Brit.] Med. Research Council, Spec. Rpt. Ser. No. **12**, 1–74.

(201) McIVER, M. A., WHITE, J. C., AND LAWSON, G. M. 1929 The rôle of the *Bacillus welchii* in acute intestinal obstruction with ligation of the veins to the obstructed loop. Ann. Surg., **89**, 647–657.

(202) McNEE, J. W., AND DUNN, J. S. 1917 The method of spread of gas gangrene into living muscle. Brit. Med. Jour., **1**, 727–729.

(203) MADSEN, T. 1899 Ueber Tetanolysin. Zeitschr. Hyg. Infektionskrank., **32**, 214–238.

(204) MADSEN, T. 1935 I. Gas gangrene antitoxins. League Nations, Health Organ., Quart. Bull., [4] Spec. No., 1–4.

(205) MADSEN, T. 1935 IV. Anti-tetanus serum. League Nations, Health Organ., Quart. Bull., [4] Spec. No., 7–8.

(206) MANZINI, C. 1931 Richerche su particolari caratteri culturali e morfologici del bacillo del tetano. Gior. Batter. e Immunol., **7**, 771–788.

(207) MARRACK, J. R. 1934 The chemistry of antigens and antibodies. [Gt. Brit.] Med. Research Council, Spec. Rpt. Ser., No. **194**, 1–135.

(208) MARSH, H., TUNNICLIFF, E. A., AND JUNGHERR, E. 1932 Types of *Clostridium welchii* found in dysentery of lambs. Jour. Infect. Diseases, **51**, 330–335.

(209) MARSH, H., WELCH, H., AND JUNGHERR, E. 1928–29 Blackleg in sheep. Jour. Am. Vet. Med. Assoc., **74**, 63–88.

(210) MASON, J. H. 1933 *B. welchii*, the "lamb dysentery bacillus," and *B. paludis:* an examination of single cell cultures. Vet. Jour., **89**, 225–231.

(211) MASON, J. H. 1935 The bloedpens strain of *Cl. welchii*, type B, Wilsdon (the "lamb dysentery bacillus"). Onderstepoort Jour. Vet. Sci. Anim. Ind., **5**, 363–389.

(212) MASON, J. H. 1935 The production of immunity against *Cl. welchii*, Type B, Wilsdon (the "lamb dysentery bacillus"). Onderstepoort Jour. Vet. Sci. Anim. Ind., **5**, 391–416.

(213) MASON, J. H. 1936 Bloedpens (lamb dysentery) in South Africa. Intern. Soc. Microbiol., 2nd Cong., 493.

(214) MASON, J. H. 1936 The toxin of *Cl. chauvoei*. Intern. Soc. Microbiol., 2nd Cong., 524.

(215) MASON, J. H., AND GLENNY, A. T. 1928 The *in vitro* titration of *B. welchii* antitoxin by its antihaemolytic power. Jour. Path. Bact., **31**, 629–632.

(216) MASON, J. H., ROBINSON, E. M. 1935 The antigenic components of the toxins of *Cl. botulinum* types C and D. Onderstepoort Jour. Vet. Sci. Anim. Ind., **5**, 65–75.

(217) MASON, J. H., ROSS, H. E., AND DALLING, T. 1931 *B. paludis* (McEwen)—a comparison with the lamb dysentery bacillus. Jour. Comp. Path. Therap., **44**, 258–265.

(218) MEISEL, H. 1936 Polysaccharides de la souche *Amylobacter*. Compt. rend. soc. biol. (Paris), **123**, 173–176.

(219) MELENEY, F. L., HUMPHREYS, F. B., AND CARP, L. 1927 An unusual fatal operative wound infection yielding a pathogenic anaerobe of the gas gangrene group not hitherto described. Surg., Gynecol. Obstet., **45**, 775–789.

(220) MEYER, K. F. 1915 The etiology of "symptomatic anthrax" in swine. "Specific gas-phlegmon of hogs." Jour. Infect. Diseases, **17**, 458–496.

(221) MEYER, K. F. 1928 Botulismus. Kolle, W., Krause, R., und Uhlenhuth, P., *Handbuch der pathogenen Mikroorganismen*. 3 Aufl., **4**, 1269–1364.

(222) MEYER, K. F. 1936 Recent studies on botulism. Intern. Soc. Microbiol., 2nd Cong., 172.

(223) MEYER, K. F., AND GUNNISON, J. B. 1929 European strains of *Cl. botulinum*. XXXVI. Jour. Infect. Diseases, **45**, 96–105.

(224) MEYER, K. F., AND GUNNISON, J. B. 1929 South African cultures of *Clostridium botulinum* and *parabotulinum*. XXXVII (with a description of *Cl. botulinum* Type D, N. Sp.). Jour. Infect. Diseases, **45**, 106–118.

(225) MIESSNER, —. 1919 Zu dem Artikel "Spontaner Rauschbrand der Schafe" von G. Witt, D.t.W. 1919, S. 171. Deut. tierärztl. Wchnschr., **27**, 185.

(226) MIESSNER, H., UND MEYN, A. 1926 Vergleichende Untersuchungen über den Rinder- und Schafrauschbrand. Deut. tierärztl. Wchnschr., **34**, 571–580.

(227) MIESSNER, H., MEYN, A., UND SCHOOP, G. 1931 Die Bradsot der Schafe. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **120**, 257–290.

(228) MITA, T. 1934 Untersuchungen über den *Bacillus histolyticus*. Japan. Jour. Expt. Med., **12**, 285–297.

(229) MONTGOMERIE, R. F., AND ROWLANDS, W. T. 1933 Pulpy kidney disease of young lambs in North Wales. The toxicity of intestinal contents and the nature of the toxic element. Vet. Jour., **89**, 388–398.

(230) MONTGOMERIE, R. F., AND ROWLANDS, W. T. 1934 Pulpy kidney disease of young lambs in North Wales—bacteria of Welch group isolated from intestinal contents. Vet. Jour., **90**, 399–403.

(231) MONTGOMERIE, R. F., AND ROWLANDS, W. T. 1936 *Cl. welchii*, Type C, Wilsdon (*B. paludis*, McEwen) isolated from sheep in North Wales. Vet. Rec., n. s. **16**, 829–832.

(232) MONTGOMERIE, R. F., AND ROWLANDS, W. T. 1936 The efficiency of commercial lamb dysentery serum. Vet. Rec., n. s. **16**, 1481–1483.

(233) MORTON, J. J., AND STABINS, S. J. 1928 Relation of *Bacillus welchii* antitoxin to the toxemia of intestinal obstruction. Arch. Surg., **17**, 860–871.

(234) NELSON, C. I. 1933 Flatulent diarrhea due to *Clostridium welchii*. Jour. Infect. Diseases, **52**, 89–93.

(235) NICHOLSON, J. A. 1934–35 Some observations on the physiological actions of *Cl. welchii* toxins. Univ. of Cambridge, Inst. of Anim. Path., 4th Rpt. of the Director, 212–226.

(236) NICOLLE, M., CÉSARI, E., ET RAPHAEL, A. 1915 Études sur le *Vibrion septique* et le *"Bacterium chauvoei."* Ann. inst. Pasteur., **29**, 165–177.

(237) NICOLLE, C., ET TRENEL, M. 1902 Recherches sur le phénomène de l'agglutination. Variabilité de l'aptitude agglutinative et de la fonction agglutinogène.—Leurs relations entre elles; leurs rapports avec la mobilité des microbes. Ann. inst. Pasteur, **16**, 562–586.

(238) OKUDA, K. 1922 On a soluble toxin produced by *Bacillus chauvoei*. Jour. Japan. Soc. Vet. Sci., **1**, 157–197, 198–199 (Eng. abstr.).

(239) ORR, J. H., JOSEPHSON, J. E., BAKER, M. C., AND REED, G. B. 1933 Variation in *Clostridium welchii*. Can. Jour. Research, **9**, 350–359.

(240) ORR, J. H., AND REED, G. B. 1933 Variation in *Cl. welchii*. Jour. Bact., **25**, 96–97.

(241) ORR, P. F. 1920 Some observations on the biological characteristics of *Bacillus botulinus* and its toxins. Abstracts Bact., **4**, 10.

(242) OURANOFF, A. 1917 Sur l'hémotoxine du *B. Welchi* (*B. perfringens*). Compt. rend. soc. biol. (Paris), **80**, 706–708.

(243) OXER, D. T. 1931 Black disease in Tasmania. Jour. Council Sci. Ind. Research, **4**, 36–44.

(244) OXER, D. T. 1932 "Pulpy kidney": or acute infectious entero-toxaemia of sucking lambs due to *B. ovitoxicus* (Bennetts). Austral. Inst. Sci. Ind., Pamphlet No. **35**, 9–25.

(245) PASTERNACK, J. G., AND BENGTSON, I. A. 1936 The experimental pathology and pathologic histology produced by the toxin of *Vibrion septique* in animals. U. S. Pub. Health Serv., Natl. Inst. Health Bull. No. **168**, 1–46.

(246) PFEIFFER, R., UND BESSAU, G. 1917 Ueber bakteriologishe Befunde bei den Gasphlegmonen Kriegsverletzter. Deut. med. Wchnschr., **43**, 1217–1220; 1255–1258; 1281–1285.

(247) PFEILER, W. 1917–18 Die Erkennung der bakteriellen Infektionskrankheiten mittels der Präzipitationsmethode. Zeitschr. Infektionskrank. Hyg. Haustiere, **19**, 153–191; 242–285.

(248) PFENNINGER, W. 1924 Toxico-immunologic and serologic relationship of *B. botulinus*, type C, and *B. parabotulinus*, "Seddon." XXII. Jour. Infect. Diseases, **35**, 347–352.

(249) PLAUT, H. C. 1917 Agglutinationstechnik und Wert der Dunkelfeldbeleuchtung beim Studium der Anaërobier. Deut. med. Wchnschr., **43**, 302.

(250) POMMERENKE, W. T. 1936 Effect of injection of bacterial filtrate on Brown-Pearce rabbits epithelioma. Jour. Am. Med. Assoc., **106**, 1654–1657.

(251) POWELL, H. M. 1923 A biological study of the diphtheria bacillus. III. The property of agglutination in pure-line strains derived from a common parent-cell. Am. Jour. Hyg., **3**, 362–369.

(252) PRIGGE, R. 1936 Experimentelle Untersuchungen über die serologischen Eigenschaften des Fraenkelschen Gasbazillus (*B. perfringens*). Zeitschr. Immunitätsf., **89**, 477–483.

(253) PRIGGE, R. 1936 Experimentelle Untersuchungen über die serologischen Eigenschaften des Fraenkel'schen Gasbazillus (*B. perfringens*). Intern. Soc. Microbiol., 2nd Cong., 175–178.

(254) PRIGGE, R. 1937 Experimentelle Untersuchungen über die serologischen Eigenschaften des Fraenkelschen Gasbazillus (*B. perfringens*). Zeitschr. Immunitätsf., **91**, 457–469.

(255) RAMON, G. 1937 L'immunité et l'influence des "substances adjuvantes et stimulantes" injectées en mélanges avec l'antigène. Introduction à une étude d'ensemble. Rév. Immunol., **3**, 193–201.

(256) RAMON, G., LEMETAYER, E., ET RICHOU, R. 1937 De l'activité immunisante des mélanges d'anatoxine et de substances adjuvantes. Rév. Immunol., **3**, 202–218.

(257) RAMON, G., LEMETAYER, E., RICHOU, R., ET NICOL, L. 1937 La toxicité et l'activité immunisante des toxines injectées a l'animal d'expériences en mélange avec des substances diverses. Rév. Immunol., **3**, 285–318.

(258) REYMANN, G. C. 1927 Untersuchungen über Tetanospasmin und Tetanolysin. Zeitschr. Immunitätsf., **50**, 31–70; 405–461; **51**, 1–26.

(259) RIS, C. 1936 Anaerobe sporevormende bacterien in verontreinigd water, in het bijzonder *Clostridium welchii*. Thesis, Amsterdam, 108 pp.

(260) ROBERTS, R. S. 1931 Ovine and bovine strains of *B. chauvoei*. Jour. Comp. Path. Therap., **44**, 246–251.

(261) ROBERTS, R. S. 1933 The practical value of the heat-stable antigen of *C. chauvoei* as an immunising agent. Jour. Comp. Path. Therap., **46**, 56–61.

(262) ROBERTSON, M. 1915–16 Notes upon certain anaerobes isolated from wounds. Jour. Path. Bact., **20**, 327–349.

(263) ROBERTSON, M. 1917–18 Notes on the occurrence of *B. tetani* in wounds. Trans. Roy. Soc. Trop. Med. Hyg., (London), **11**, 56–68.

(264) ROBERTSON, M. 1919–20 Serological groupings of *Vibrion septique* and their relation to the production of toxin. Jour. Path. Bact., **23**, 153–170.

(265) ROBERTSON, M. 1929 The organisms associated with gas gangrene. [Gt. Brit.] Med. Research Council, System Bact., No. **3**, 225–297.

(266) ROBERTSON, M., AND FELIX, A. 1930 Serological studies in the group of the spore-bearing anaërobes. II. *In vivo* experiments with an "O" immune serum to *Vibrion septique* devoid of antitoxin content. Brit. Jour. Expt. Path., **11**, 14–23.

(267) ROBINSON, E. M. 1929 Notes on botulism in the domesticated animals. Union of So. Africa, Dept. of Agr., 15th Rpt. of the Director of Vet. Serv. and Anim. Ind., 97–110.

(268) ROBINSON, E. M. 1930 The bacteria of the *Clostridium botulinum* C and D types. Union of So. Africa, Dept. of Agr., 16th Rpt. of the Director of Vet. Serv. and Anim. Ind., 107–142.

(269) ROSE, A. L., AND EDGAR, G. 1936 Entero-toxaemic jaundice of sheep and cattle. A preliminary report on the aetiology of the disease. Austral. Vet. Jour., **12**, 212–220.

(270) ROTTGARDT, A. 1929 Diagnostischer Wert der Präzipitations- und Agglutinationsverfahren beim Rauschbrand. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **113**, 245–256.

(271) ROUX, E. 1888 Immunité contre le charbon symptomatique conférée par des substances solubles. Ann. inst. Pasteur, **2**, 49–53.

(272) RUSCH, H. P., AND PRESTON, W. S. 1936 The effect of filtrates of *Cl. histolyticum* upon the growth of animal tumors. Proc. Soc. Expt. Biol. Med., **34**, 581–584.

(273) SABRAZÈS, J., ET RIVIÈRE, P. 1897 Réaction agglutinante du sérum de l'homme et de l'animal tétaniques sur le bacille de Nicolaïer. Compt. rend. soc. biol. (Paris), **49**, 618–620.

(274) SACQUÉPÉE, E. 1915 A propos d'une note de MM. M. Weinberg et P. Séguin, intitulée: "Le *B. oedematiens* et la gangrène gazeuse.' 'Compt. rend. soc. biol. (Paris), **78**, 547–550.

(275) SACQUÉPÉE, E. 1915 Le bacille de l'oedème gazeux malin. Compt. rend. soc. biol. (Paris), **78**, 316–318.

(276) SACQUÉPÉE, E. 1916 Sur le bacille de l'oedème gazeux malin (observations à propos d'une note de MM. Weinberg et Séguin). Compt. rend. soc. biol. (Paris), **79**, 115–116.

(277) SACQUÉPÉE, E. 1917 Sur le *Bacillus bellonensis* (ancien Bacille de l'oedème gazeux malin). Préparation de sérums specifiques; quelques propriétés essentielles des sérums. Compt. rend. soc. biol. (Paris), **80**, 850–852.

(278) SALKOWSKI, E. 1898 Ueber die Wirkung der Antiseptica auf Toxine. Berl. klin. Wchnschr., **35**, 545–549.

(279) Salmonella Subcommittee of the Nomenclature Committee of the International Society for Microbiology 1934 The genus *Salmonella* Lignières, 1900. Jour. Hyg., (London) **34**, 333–350.

(280) SCHNAYERSON, A. F., AND SAMUELS, S. L. 1930 The relationship of two hemotoxic antigens in *B. welchii* growth products. Jour. Immunol., **18**, 141–151.

(281) SCHOENHOLZ, P., AND MEYER, K. F. 1925 Studies on the serologic classification of *B. botulinus*. II. Agglutination. Jour. Immunol., **10**, 1–53.

(282) SCHÜTZE, H. 1921 The permanence of the serological paratyphoid B types, with observations on the non-specificity of agglutination with "rough" variants. Jour. Hyg., (London) **20**, 330–341.

(283) SCOTT, J. P. 1925 Studies on certain characteristics of *Clostridium chauvoei* and *Clostridium edematis*. Jour. Bact., **10**, 265–313.

(284) SCOTT, J. P. 1926 A comparative study of strains of *Clostridium chauvoei* obtained in the United States and abroad. Jour. Infect. Diseases, **38**, 262–272.

(285) SCOTT, J. P. 1928 The etiology of blackleg and methods of differentiating *Clostridium chauvoei* from other organisms found in cases of blackleg. Cornell Vet., **18**, 259–271.

(286) SCOTT, J. P., TURNER, A. W., AND VAWTER, L. R. 1934 Gas edema diseases. Twelfth Intern. Vet. Cong., **2**, 168–187.

(287) SEDDON, H. R. 1922 Bulbar paralysis in cattle due to the action of a toxicogenic bacillus, with a discussion on the relationship of the condition to the forage poisoning (botulism). Jour. Comp. Path. Therap., **35**, 147–190.

(288) SEDDON, H. R. 1922 The specific identity of *Bacillus parabotulinus*. Jour. Comp. Path. Therap., **35**, 275–280.

(289) SHIRAISHI, S. 1931 Studien über Welch-Fraenkel'sche Bacillen mit besonderen Berücksichtigung ihrer Toxine und Antitoxine. Japan. Jour. Expt. Med., **9**, 613–618.

(290) SIEBENMANN, C. 1933 Refractometric examination of purified antitoxins. Biochem. Jour., **27**, 1745–1752.

(291) SIMONDS, J. P. 1915 Studies in *Bacillus welchii*, with special reference to classification and to its relation to diarrhea. Rockefeller Inst. Med. Research Monog., **5**, 1–130.

(292) SMITH, L. 1937 Some serological aspects of the S-R change in *Clostridium histolyticum*. Jour. Bact., **34**, 409–417.

(293) SNIPE, P. T., AND SOMMER, H. 1928 Studies on botulinus toxin. 3. Acid precipitation of botulinus toxin. Jour. Infect. Diseases, **43**, 152–160.

(294) SNYDER, M. L. 1936 Mechanism of the non-specific serum agglutination of the obligate anaerobes *Clostridium paraputrificum* and *Clostridium capitovalis*. Jour. Bact., **32**, 411–422.

(295) SNYDER, M. L. 1936 The serologic agglutination of the obligate anaerobes *Clostridium paraputrificum* (Bienstock) and *Clostridium capitovalis* (Snyder and Hall). Jour. Bact., **32**, 401–410.

(296) SNYDER, M. L. 1937 Further studies on *Bacillus difficilis* (Hall and O'Toole). Jour. Infect. Diseases, **60**, 223–231.

(297) SNYDER, M. L., AND HALL, I. C. 1935–36 *Bacillus capitovalis*, a new species of obligate anaerobe encountered in *post mortem* materials, in a wound infection, and in the feces of infants. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **135**, 290–297.

(298) SOMMER, H. 1936–37 Purification of botulinus toxin. Proc. Soc. Expt. Biol. Med., **35**, 520–521.

(299) SOMMER, H., NEALON, P. J., AND SNIPE, P. T. 1928 Studies on botulinus toxin. 4. Dialysis experiments. Jour. Infect. Diseases, **43**, 161–166.

(300) SOMMER, H., AND SNIPE, P. T. 1928 Studies on botulinus toxin. 2. Further purification by adsorption on colloids. Jour. Infect. Diseases, **43**, 145–151.

(301) SORDELLI, A. 1923 Flore anaérobie de Buenos-Aires. Compt. rend. soc. biol. (Paris), **89**, 53–55.

(302) SORDELLI, A., ET FERRARI, J. 1931 Quelques propriétés du sérum antimicrobien contre le *Bacillus perfringens*. Compt. rend. soc. biol. (Paris), **106**, 141–142.

(303) SPAULDING, E. H., AND RETTGER, L. F. 1937 The *Fusobacterium* genus. I. Biochemical and serological classification. Jour. Bact., **34**, 535–548.

(304) SPRAY, R. S. 1936 Semisolid media for cultivation and identification of the sporulating anaerobes. Jour. Bact., **32**, 135–155.

(305) STARIN, W. A., AND DACK, G. M. 1923 Agglutination studies of *Clostridium botulinum*. Jour. Infect. Diseases, **33**, 169–183.

(306) STARIN, W. S., AND DACK, G. M. 1924 Complement-fixation studies on *Clostridium botulinum*. Jour. Infect. Diseases, **34**, 137–147.

(307) STEWART, M. W., AND WEST, R. 1916 Study of a strain of *B. welchii* isolated in France together with some notes on gastric ulcers. Jour. Immunol., **1**, 189–199.

(308) STEWART, S. E. 1932 Determination of the L_0 and the L_+ dose of botulinus toxin by complement fixation. Jour. Infect. Diseases, **51**, 324–329.

(309) STEWART, S. E. 1936 Studies on the production of toxin by *Clostridium histolyticum*. U. S. Pub. Health Rpts., **51**, 1272–1279.

(310) STEWART, S. E. 1938 The titration of *bifermentans* antitoxin and the relationship between *Clostridium bifermentans* and *Clostridium sordellii*. Jour. Bact., **35**, 13–14.

(311) SVARTZ, N. 1927 Étude sur les bactéries intestinales iodophiles et spécialement sur les clostridies iodophiles. Acta Med. Scand., Sup. **20**, 1–149.

(312) TANI, S. 1934 Studies on botulinum toxin. (First report.) Japan. Jour. Expt. Med., **12**, 9–31.

(313) TANI, S. 1934 Studies on botulinum toxin. (Second report.) On the toxin produced in biuret-free media and its characteristics. Japan. Jour. Expt. Med., **12**, 33–47.

(314) TEMPÉ, G., ET GRABAR, J. 1930 Bacille II de Ghon et Sachs dans une cholécystite gangréneuse. Compt. rend. soc. biol. (Paris), **103**, 1149–1151.

(315) THEILER, A. 1920 Cause and prevention of lamziekte. Union of So. Africa, Dept. Agr. (cited by Robinson, E. M., 1930).

(316) THEILER, A., UND ROBINSON, E. M. 1927 Der Botulismus der Haustiere. Definition, Historisches, Literatur-Übersicht, Experimentelles. Zeitschr. Infektionskrank. Hyg. Haustiere, **31**, 165–220.

(317) THEILER, A., AND ROBINSON, E. M. 1928 Botulism (parabotulism) in equines. Union of So. Africa, Dept. of Agr., 13th and 14th Rpts. of the Director of Vet. Educ. and Research, 47–68.

(318) THEILER, A., ET ROBINSON, E. M. 1937 Parabotulisme des équidés. Rev. gén. méd. vét., **36**, 193–199.

(319) THEILER, A., in collaboration with VILJOEN, P. R., GREEN, H. H., TOIT, P. J. DU, MEIER, H., AND ROBINSON, E. M. 1927 Lamsiekte (parabotulism) in cattle in South Africa. Union of So. Africa, Dept. of Agr., 11th and 12th Rpts. of the Director of Vet. Educ. and Research, 821–1361.

(320) THURSTON, H. F. 1931 The rôle of toxin of *Bacillus welchii* in the toxemia of acute intestinal obstruction. An experimental study. Arch. Surg., **22**, 72–85.

(321) TOPLEY, W. W. C., AND WILSON, G. S. 1936 *The principles of bacteriology and immunity*. Wood, Baltimore, 1645 pp.

(322) TOWNSEND, C. T. 1929 Comparative study of nontoxic and toxic strains of *Clostridium parabotulinum*. XXXV. Jour. Infect. Diseases, **45**, 87–95.

(323) TSCHERTKOW, L. 1929 Ueber die Eigenschaften des Tetanustoxins. Zeitschr. Immunitätsf., **63**, 262–276.

(324) TULLOCH, W. J. 1917 On the bacteriology of wound infections in cases of tetanus and the identification of *Bacillus tetani* by serological reactions. Jour. Roy. Army Med. Corps, **29**, 631–661.

(325) TULLOCH, W. J. 1918 The isolation and serological differentiation of *Bacillus tetani*. Proc. Roy. Soc. (London), Ser. B, **90**, 145–158.

(326) TULLOCH, W. J. 1919 Report of bacteriological investigation of tetanus carried out on behalf of the war office committee for the study of tetanus. Jour. Hyg. (London), **18**, 103–202.

(327) TULLOCH, W. J. 1927 A critical review of the present position of bacterial agglutination. Jour. Roy. Army Med. Corps, **48**, 96–116; 192–203; 267–285; 358–368; 435–447.

(328) TURNER, A. W. 1930 Black disease (infectious necrotic hepatitis) of sheep in Australia: a toxaemia induced by a specific bacterium (*B. oedematiens*) in hepatic lesions resulting from the migration of young liver flukes (*F. hepatica*). Austral. Council Sci. Ind. Research Bull. No. **46**, 1–141.

(329) VAILLARD, L. 1892 Sur quelques points concernants l'immunité contre le tétanos. Ann. inst. Pasteur, **6**, 224–232.

(330) VAUCHER, E. 1917 Le *B. oedematiens* dans un cas de gangrène gazeuse. Compt. rend. soc. biol. (Paris), **80**, 251–253.

(331) VAWTER, L. R., AND RECORDS, E. 1925–26 Recent studies on icterohemoglobinuria of cattle. Jour. Am. Vet. Med. Assoc., **68**, 494–513.

(332) VAWTER, L. R., AND RECORDS, E. 1929 Immunization of cattle against bacillary hemoglobinuria. Jour. Am. Vet. Med. Assoc., **75**, 201–214.

(333) VAWTER, L. R., AND RECORDS, E. 1931 Serologic study of sixteen strains of *Bacillus hemolyticus*. Jour. Infect. Diseases, **48**, 581–587.

(334) WAGNER, E. 1924 Biochemical activities of *B. botulinus*, type C, and B. *parabotulinus*, "Seddon." XXIII. Jour. Infect. Diseases, **35**, 353–360.

(335) WALBUM, L. E. 1909 Studien über Toxinbildung. Zeitschr. Immunitätsf., **3**, 70–97.

(336) WALBUM, L. E., AND REYMANN, G. C. 1933 The production of toxins by *Clostridium welchii*. Jour. Path. Bact., **36**, 469–483.

(337) WALBUM, L. E., AND REYMANN, G. C. 1936 Memorandum concerning an international standard for gas-gangrene antitoxin (*histolyticus*). League Nations, Health Organ., Quart. Bull. [5] Spec. No., 690–694.

(338) WALBUM, L. E., AND REYMANN, G. C. 1936 The production of toxin by *Bacillus oedematis maligni* (*Vibrion septique*). Jour. Path. Bact., **42**, 351–361.

(339) WALBUM, L. E., AND REYMANN, G. C. 1937 The production of toxin by *Clostridium oedematiens* (*B. novyi*). Jour. Path. Bact., **44**, 379–387.

(340) WEIL, L., AND KOCHOLATY, W. 1937 Studies on the proteinase of *Clostridium histolyticum*. Biochem. Jour., **31**, 1255–1267.

(341) WEINBERG, M. 1936 Des holosérums. Intern. Soc. Microbiol., 2nd Cong., 493–494.

(342) WEINBERG, M. 1936 Le perfringens en pathologie animale. Congr. intern. path. compar. 3e Cong., **2** Sect. méd. vét., 406–421.

(343) WEINBERG, M. 1936 Opener: Récent progrès dans l'étude des microbes anaérobies. Intern. Soc. Microbiol., 2nd Cong., 167–170.

(344) WEINBERG, M., ET BAROTTE, J. 1928 Synergie des anticorps. Ann. inst. Pasteur, **42**, 619–641.

(345) WEINBERG, M., ET BAROTTE, J. 1929 Préparation des sérums antigangréneux monovalents avec des toxines formolées. Ann. inst. Pasteur, **43**, 453–536.

(346) WEINBERG, M. ET BAROTTE, J. 1929 Toxines du *Bacillus perfringens* et sérums anti-*perfringens*. Compt. rend. soc. biol. (Paris), **100**, 733–734.

(347) WEINBERG, M., ET COMBIESCO, N. 1930 Lésions hémorragiques causées par le *B. perfringens*. Ann. inst. Pasteur, **45**, 547–580.

(348) WEINBERG, M., DAVESNE, J., ET HABER, P. 1932 Les agglutinogènes du *Vibrion septique*. Valeur comparative du sérum anti-O et du sérum antitoxique. Ann. inst. Pasteur, **49**, 303–318.

(349) WEINBERG, M., DAVESNE, J., MIHAILESCO, M., ET SANCHEZ, C. 1929 Etude comparative du *Vibrion septique* et du *Bacillus chauvoei* par la réaction de fixation. Compt. rend. soc. biol. (Paris), **101**, 907–909.

(350) WEINBERG, M., ET GINSBOURG, B. 1927 *Données récentes sur les microbes anaerobies et leur rôle en pathologie*. Masson et Cie., Paris, 291 pp.

(351) WEINBERG, M., ET GOY, P. 1925 Emploi de l'anatoxine dans la préparation du sérum antibotulinique. Compt. rend. soc. biol. (Paris), **92**, 564–565.

(352) WEINBERG, M., ET GUILLAUMIE, M. 1936 Holoserum anti-*perfringens*. Compt. rend. soc. biol. (Paris), **122**, 1224–1227.

(353) WEINBERG, M., ET GUILLAUMIE, M. 1936 Les microbes du groupe *perfringens*. Holoserum anti-*perfringens*. Compt. rend. soc. biol. (Paris), **121**, 1275–1278.

(354) WEINBERG, M., ET GUILLAUMIE, M. 1936 Titres apparents et titres réels des sérums antitoxiques. Compt. rend. soc. biol. (Paris), **123**, 661–664.

(355) WEINBERG, M., GUILLAUMIE, M., ET FORGEOT, P. 1935 Premier cas d'infection à *B. paludis* observé chez le mouton, en Turquie. Bull. acad. vét. France, n. s. **8**, 505–507.

(356) WEINBERG, M., GUILLAUMIE, M., ET STAUB, A. 1935 Premier cas d'infection à *B. paludis* observé chez le mouton, en France. Bull. acad. vét. France, n. s. **8**, 502–504.

(357) WEINBERG, M., ET NASTA, M. 1920 Rôle des hémolysines dans l'intoxication microbienne. Ann. inst. Pasteur, **34**, 690–700.

(358) WEINBERG, M., NATIVELLE, R., ET PRÉVOT, A. R. 1937 *Les microbes anaérobies*. Masson et Cie, Paris, 1186 pp.

(359) WEINBERG, M., ET SÉGUIN, P. 1915 Le *B oedematiens* et la gangrène gazeuse. Compt. rend. soc. biol. (Paris), **78**, 507–512.

(360) WEINBERG, M., ET SÉGUIN, P. 1915 Flore microbienne de la gangrène gazeuse. Compt. rend. soc. biol. (Paris), **78**, 686–689.

(361) WEINBERG, M., ET SÉGUIN, P. 1915 Réponse a M. Sacquépée. Compt. rend. soc. biol. (Paris), **78**, 550–551.

(361) WEINBERG, M., ET SÉGUIN, P. 1917 Démonstration de lésions provoquées chez le cobaye, par le *B. histolyticus*. Quelques observations sur la toxine de ce microbe. Compt. rend. soc. biol. (Paris), **80**, 157–159.

(363) WEINBERG, M., ET SÉGUIN, P. 1918 Un cas de gangrène gazeuse a *B. oedematiens* chez un civil. Compt. rend. soc. biol. (Paris), **81**, 184–185.

(364) WEISS, J. E., AND RETTGER, L. F. 1937 The Gram-negative *Bacteroides* of the intestine. Jour. Bact., **33**, 423–434.

(365) WHITE, P. B. 1937 Remarks on bacterial taxonomy. Zentr. Bakt. Parasitenk., 2 Abt., **96**, 145–149.

(366) WILLIAMS, B. W. 1927 Hunterian Lecture on the importance of toxaemia due to anaerobic organisms in acute intestinal obstruction and peritonitis. Lancet (London), **1**, 907–912.

(367) WILSDON, A. J. 1931 Observations on the classification of *Bacillus welchii*. Univ. Cambridge, Inst. Anim. Path., 2nd Rpt. of the Director, 53–85.

(368) WILSDON, A. J. 1932–33 The relationship of "*Bacillus ovitoxicus*" (Bennetts) to the *Clostridium welchii* group. Univ. Cambridge, Inst. Anim. Path., 3rd Rpt. of the Director, 46–51.

(369) WILSON, G. S. 1937 Abstract of paper of: McCLUNG, L. S. Studies on anaerobic bacteria. X. Heat stable and heat labile antigens in the botulinus and related groups of spore-bearing anaerobes. (Jour. Infect. Dis., **60**, 122–8). Bull. Hyg., **12**, 388.

(370) WITT, —. 1919 Spontaner Rauschbrand der Schafe. Scher-, Gebärmutter- und Euter-Brand. Deut. tierärztl. Wchnschr., **27**, 171–172.

(371) WOLMAN, I. J. 1937 The preservation of tetanus toxin by the lyophile process. Science, n. s. **86**, 382.

(372) WOLTERS, K. L., UND DEHMEL, H. 1928 Zur Züchtung und Differenzierung der anaëroben Sporenbildner unter besonderer Berücksichtigung der Rauschbrand- und Pararauschbrandbazillen. Zentr. Bakt. Parasitenk., 1 Abt. Orig., **108**, 264–275.

(373) WOODRUFF, H. A. 1936 Some toxaemias of animals due to anaerobic organisms. A comparative pathological study of recent work with particular reference to the toxaemia of intestinal obstruction in man. Brit. Med. Jour., **1**, 406–409.

(374) WUTH, O. 1923 Serologische und biochemische Studien über das Hämolysin des Fränkelschen Gasbrandbazillus. Biochem. Zeitschr., **142**, 19–28.

(375) ZEISSLER, J. 1918 Über die Reinzüchtung pathogener Anaërobier. Zeitschr. Hyg. Infektionskrank., **86**, 52–66.

(376) ZEISSLER, J. 1919 Der Rauschbrand und verwandte Erkrankungen der Tiere. Berl. klin. Wchnschr., **56**, 107–110.

(377) ZEISSLER, —. 1924 ———————. Deut. tierärztl. Wchnschr., **32**, 628–629.

(378) ZEISSLER, J. 1928 Die Gasödeminfektionen des Menschen. Kolle, W., Krause, R., und Uhlenhuth, P. *Handbuch der pathogenen Mikroorganismen*, 3 Aufl., **4**, 1097–1212.

(379) ZEISSLER, J., UND RASSFELD, L. 1928 Die anaerobe Sporenflora der europäischen Kriegsschauplätze 1917. Veröffentl. Kriegs- und Konstitutionspathologie, **5**, Heft 2, 1–99.

(380) ZEISSLER, J., UND RASSFELD, L. 1929 Der *Bacillus gigas*. Arch. wiss. prakt. Tierheilk., **59**, 419–443.

(381) ZELLER, —. 1927 Untersuchungen über Rauschbrand. Deut. tierärztl. Wchnschr., **35**, 259–260.