

that no definite evidence is at hand to indicate that the forms were not small when, during Pleistocene time, the distribution of these types extended westward into the region now occupied by the Channel Islands, unless that evidence rests on the failure to identify similar forms in Pleistocene deposits on the mainland.

The presence of Pleistocene elephants on Santa Rosa Island furnishes apparently a striking confirmation of the view that profound changes in the coast line of southern California have occurred in late geological time.

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#### A COLLECTION OF ANAEROBIC BACTERIA

DURING the past sixteen years I have devoted much time to collecting, identifying and studying anaerobic bacteria. My interest in these micro-organisms grew out of the fact that all the bacteriologists whom I knew in 1912 threw up their hands when anaerobic bacteria were mentioned; not one understood how to isolate an obligate anaerobe from a mixed culture with any degree of precision or how to identify one afterward. In the same year I was fortunate enough to be assigned the task of developing a tetanus antitoxin laboratory for the Cutter Biological Laboratory in Berkeley, California. Tetanus antitoxin had never been made anywhere in the western United States previously, and this laboratory was the first to undertake it. During the following year, about the time we had successfully completed the immunization of our first horses, I met Dr. K. F. Meyer, who had just been appointed associate professor of bacteriology in the University of California. Dr. Meyer was the first bacteriologist of my acquaintance who seemed to know anything about the obligate anaerobes, and while I have never been directly associated with him in his laboratory, notwithstanding he was both my predecessor and successor at Berkeley, I am glad to acknowledge his valuable guidance on numerous occasions.

The early phase of my studies involved securing so-called "type cultures" from as many sources as possible, and the development of my own technic in checking the purity and identity of these. A few of these cultures were pure and correctly named, but some of them were impure and many were misnamed. It was not until Weinberg and Seguin's memorable monograph "La Gangrene Gazeuse" (1917) became available in 1920 that we were able to recognize with any degree of certainty any but a few of the commonest species in the collection, which then numbered sixty-three strains.

I have continued to add new strains to the collection, partly by accretion from other laboratories, but mainly by isolation from original materials, soil, water, milk, accidental contaminations of culture media, spontaneous infections in laboratory animals, and particularly during the past five years from human sources, both ante- and post-mortem.

The collection now contains 319 strains, 282 of which have been at least tentatively purified and identified, while thirty-seven other non-pathogenic strains have been presumably purified but not identified. Some of the latter possibly represent new species, others are probably species whose descriptions are buried in obscure or unavailable scientific articles. These obviously offer excellent material for further taxonomic research in this group.

Among those that have been identified are the following species, those that are definitely pathogenic being starred thus \*. In a few instances the identification is only tentative.

B. bifermentans .....	6 strains
*B. botulinus, Type A .....	4 "
*B. botulinus, Type B .....	4 "
B. butylicus, B. amylobacter, B. butyricus and related species .....	9 "
B. centrosporogenes .....	28 "
*B. chauvoei .....	4 "
B. fallax .....	1 "
B. flabelliferum .....	4 "
*B. hemolyticus .....	1 "
*B. histolyticus .....	13 "
B. multifementans .....	9 "
B. non-fermentans .....	2 "
*B. Novyi .....	8 "
*B. oedematoides .....	3 "
B. putrefaciens .....	4 "
B. putrificus (and B. cochlearius?) .....	8 "
*B. septicus .....	14 "
*B. Sordellii .....	3 "
B. sporogenes .....	32 "
B. sphenoides .....	1 "
B. sub-terminalis .....	1 "
B. tertius .....	15 "
*B. tetani .....	24 "
B. tetanomorphus .....	3 "
B. tyrosinogenes .....	1 "
*B. Welchii .....	40 "
Fusiformis (Sp.?) .....	3 "
M. gazogenes .....	37 "
Total identified strains .....	282

The number of strains of each species in the collection gives a clue, in a sense, to the frequency with which one who collects anaerobes in much the same spirit as an amateur entomologist collects butterflies

is apt to encounter them. Much depends, however, on the type of material being studied. When we first began collecting laboratory strains in 1913 many of them proved to be or to contain *B. sporogenes*, irrespective of label; now it is rare to receive a contaminated or misnamed culture from another laboratory. *B. sporogenes* occurs also not infrequently in soil, water and feces, but one almost never isolates it from autopsy material, except in case of a perforated intestine, and even then only rarely. Certainly the most common anaerobe recovered by us in human autopsies is *B. Welchii*, being often no doubt an agonal or post-mortem invader of the blood stream and tissues. *B. centrosporogenes* is one of the most ubiquitous and is likely to turn up in all kinds of material, both animate and inanimate. *B. tertius* and *B. histolyticus* are also fairly common in materials exposed to fecal or soil contamination. *M. gazogenes* is common in the mouth but has also been recovered elsewhere in the body.

The stock cultures are kept in brain medium<sup>1</sup> which not only serves as the medium of choice for culture of the largest number of species of anaerobes, but also excels most other media from the standpoint of viability. Most of the sporulating forms retain their viability for years sealed in brain medium, the non-sporulating forms for several months. The cultures are kept in duplicate, one tube being sealed, the other capped with "Capes-Viscose," the cotton plug having been removed in both cases. Capes Viscose does not form a perfect seal against evaporation, but it is better than rubber, which deteriorates very rapidly and permits easy access to a culture for several months. When the tube is opened, by cutting off the cap, a sterile cotton plug is inserted temporarily until the culture is capped again.

Many of the above strains have, of course, been referred to in considerable detail in various publications;<sup>2</sup> others have not been described at all, but they

offer valuable problems for research in morphology, physiology and pathogenicity. There are also interesting problems in taxonomy and nomenclature to be solved, as, for example, the possible identity of some strains designated by separate specific names, or the separation of certain strains now designated by a single name into two or more species. The questions of serologic relationships, of synergic and symbiotic action and further commercial exploitation are all worthy of more study.

It has long been the writer's custom to distribute pure, identified strains of these and other microorganisms free of charge on request from other laboratories interested in them for purposes of teaching or research. The burden of demands upon the collection has now become so great, however, that it will be necessary hereafter to make a nominal charge, similar to that imposed by the American Type Culture Collection, of which many of my strains form a part.

It is natural, perhaps, for one to feel that his hobby constitutes a neglected field in education. Yet it is still true, as it was fifteen years ago, though of course to a lesser degree, that most bacteriologists are primarily aerobic bacteriologists (we anaerobic bacteriologists only have to come up for air occasionally). Most of them are still unable to deal scientifically with any but the commonest forms in pure culture. Knowledge of satisfactory means of isolation is limited to a few, and none knows better than the writer how often the most acceptable methods fall short for particular organisms in certain mixtures; our culture media are so highly selective.

Most courses in general and medical bacteriology slight the anaerobes because the technic is naturally more difficult than that for aerobes. Courses in anaerobic bacteriology should therefore require the former as prerequisites. Such a special course was given at Dijon during the war for army technicians. The writer also gave a course in anaerobic bacteriology for several years at the University of California in Berkeley, but this, I understand, was discontinued in 1925. It is now planned to resume the course at the University of Colorado School of Medicine in Denver, during the summer session beginning June 16, 1928. There will also be facilities for a limited number of graduate students.

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