

Date: 29 July 1953

ONR: 135 660

Contract Number: N8ONR-72000

Annual Rate: \$1350

Contractor: Gonzaga University

Principal Investigator: Edw. L. Foubert Jr., Ph. D.

Title of Project: Anaerobic non-clostridial flora of the skin and/or the mucous membranes of man.

Objectives: To attempt a complete survey of the skin and/or mucous membranes of man for obligately anaerobic non-clostridial microorganisms. In the process it is hoped to work out adequate methods of culturing, identifying and classifying these microorganisms.

Abstract or Summary of Results:

In duplicate scrapings of the arm, rib, sternum, coccygeal and thigh areas of the skin of 6 men and 1 woman 230 obligate anaerobes have been isolated. The percentage of obligate anaerobes in the total anaerobic-faultiative varied from 0-20% (average 6.7%) over the ribs 5 inches below the armpit, 0-22.2% (average 2.9%) on the lateral brachial surface of the arm, 0-1.3% (average 0.3%) over the xiphoid process of the sternum, 0-20% (average 2.4%) over the coccygeal region of the spine, and 1.4-27.7% (average 6.0%) on the subinguinal area of the inner thigh 4 inches below the groove of the groin.

In a semi-annual progress of 29 January 1953 the probable relationships of these organisms, based on the available routine tests, was given. It was also indicated (1) that the available routine did not lend themselves to a satisfactory taxonomic treatment of the organisms, (2) that quantitative glucose determinations failed to help because many of the microbes obviously produced interfering aldehydes or ketones from the apparently necessarily rich medium used for their culture, and (3) that experiments on amino acid utilization as a basis for taxonomic treatment were being undertaken.

Since that time an extensive series of tests has indicated that most of these organisms give a barely demonstrable growth in a medium consisting of: yeast extract (Difco), 1.0 g; agar (Difco), 1.0 g; sodium thioglycollate (Paltimore Biological Laboratories), 1.0 g; anhydrous disodium hydrogen phosphate, 2.5 g; resazurin, 0.001 g; distilled water, 1000 ml. When utilizable amino acids, purines, or pyrimidines are added singly to this medium in a concentration of 0.1% recognizably better growth could be readily determined. The yeast extract was included as a necessary source of vitamins. Some organisms did not evidence demonstrable growth in this medium, even in the presence of any of the 25 amino acids, purines and pyrimidines tested. The amount of yeast extract necessary for growth to occur with the latter organisms produced too heavy a growth to permit detection of increased amounts in the presence of the added amino acids, purines and pyrimidines. These organisms were few, however, and were included in all experiments using the basal medium as indicated.

In a series of experiments 166 organisms were tested in duplicate for the utilization (singly) of 18 amino acids (glycine, 1 alanine, 1 aspartic acid, dl leucine 1 lysine, 1 cystine, dl methionine, 1 phenylalanine, 1 tyrosine, 1 arginine, dl tryptophane, dl isoleucine, dl valine, dl serine, 1 histidine, 1 arginine,

creatine, and 1 asparagine) and of 7 purines and pyrimidines (xanthine, uracil, allantoin, guanine, thymine, adenine and uric acid). In the process of running the above tests the ability of these organisms to utilize urea, citric acid, gelatin, or glycogen was determined under the same conditions.

As far as we have gone the results of these tests indicate that many of the previously proposed relationships were based on routine tests incapable of providing a fundamentally sound taxonomic treatment. We feel that these tests are by no means finished. Many of them need repeating for the sake of certitude. Furthermore, some preliminary work indicates that glucose and other carbohydrate utilizations can be determined in the above basal medium, thus solving one of our difficulties, that of separating the carbohydrate fermenters from the carbohydrate non-fermenters. Tentatively, we think that 15 of these organisms are related to Micrococcus activus, 16 to Micrococcus prevotii (the distinction between these two species becomes slim in itself). 11 to Micrococcus aerogenes, 12 to Micrococcus niger, 14 to Micrococcus to Micrococcus anaerobius and 1 to Micrococcus variabilis. 80 strains seem to bear no relationship to any previously described anaerobic micrococcus. In addition we have 15 strains of anaerobic streptococci, 5 of Propionibacterium acnes, and 58 of Micrococcus saccharolyticus.

It should be evident that this work is not in a satisfactorily conclusive condition and that certainly no technical publication is as yet desirable or in the best interests of bacteriology. However, publication of the final results will assuredly be done as soon as is possible. On the other hand, we realize that this ONR contract should be terminated and that such termination is being held up in lieu of a final report to the Microbiology Branch of the Office of Naval Research. We therefore ask that this summary and abstract of the work done under our ONR contract be accepted as a final report for the purpose of terminating the present contract.

In compliance with a letter of 21 January 1953 from Walter D. Smith, resident representative of the Office of Naval Research in Seattle, we are submitting to his office (a) a final property inventory, (b) certificate of compliance with patent provisions and schedule of invention disclosures submitted, (c) contractor's release of government, (d) final invoice (original and five (5) copies), (e) certificate of compliance with conditions relative to employees under the contract as set forth in Section D - Price and Payments, as amended.

Sincerely yours,

Edw. L. Foubert Jr.

Edw. L. Foubert Jr., Ph. D.
Chairman, Department of Biology

Copy to: Director
Office of Naval Research
1000 Geary Street
San Francisco 9, California