Bacterial flora of the alimentary tract of Grylloblatta campodeiformis campodeiformis Walker
by Albert L Burroughs

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of master of Science in Bacteriology at Montana State College
Montana State University

Abstract:
A study has been made of such bacteria as may be found in the alimentary tract of Grylloblatta campodeiformis campodeiformis Walker.

This insect is a cold-loving creature which lives at temperatures near freezing. With due precautions to avoid intruders, cultures were made from the alimentary tract by plating in dextrose agar. Incubations were made aerobically and anaerobically at 4°C, 18°C and 28°C. No growth was obtained anaerobically. Colonies from the aerobic plates were picked and streaked on dextrose agar. The optimum temperature for all but one culture was found to be room temperature (25°C). This one culture grew best at 17°C to 22°C.

Morphological studies of each of the cultures was made and inoculations were made into various media in an attempt to classify the strains of bacteria.

Fifty strains of organisms obtained from two specimens were included in this study. Sixteen (32 per cent) were found to be gram-positive cocci of the genus Micrococcus, 16 (32 per cent)were gram-positive rods of the genus Bacterium, and 18 (36 per cent) were gram-positive spore-formers of the genus Bacillus.

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ALBERT L. BURROUGHS

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INTRODUCTION

This investigation was undertaken as an attempt to isolate and study the bacterial flora of the alimentary tract of Grylloblatta campodeiformis campodeiformis Walker. This insect was discovered in the Canadian Rockies in 1913 by Dr. E. M. Walker. Crampton (1915) has the following to say of the insect. "Grylloblatta campodeiformis combines within itself characters common to a number of 'Orthopteroid' insects. Indeed in many respects it may be considered as a veritable living fossil, and from the point of view of the study of insect phylogeny it is one of the most important of recent pterygotan forms."

Gurney (1937) states that the distributional data regarding Grylloblatta are still very fragmentary. The evidence points to the presence of many widely separated, more or less isolated, units of population. The three forms in the pacific coast states are distinct and the natural barriers clear. Grylloblatta sculleni occurs in Oregon. Grylloblatta campodeiformis occidentalis occurs on Mt. Baker, Washington. Grylloblatta barberi occurs in the Sierra Nevada Mountains in Plumas County, California, and is separated from its nearest Cascade relative by valleys in the watershed of the Klamath River. Grylloblatta campodeiformis campodeiformis occurs at Banff, Alberta and Gallatin County Montana. Another genus of the Grylloblattidae, Galloisiana occurs in Japan. This genus is represented by two subgenera, Galloisiana and Ishiana, nipponensis being the only species of Galliosiana and represented by one adult male and two nymphs. The subgenus Ishiana is represented by one species, notabilis. A single male nymph has been taken of the latter.

Mills and Pepper, (1937) found that the temperature most desired by these insects was 3.7°C; that starting from 3.7°C, upon cooling there was a reduction in activity at 0.1°C and that this lethargic condition continued to -5.6°C. At this point there was increased activity for a short time, prostration ensuing at -6.2°C. Apparently the insect went into no dormant state before prostration. In travelling up the temperature scale from 3.7°C, the first change noted was an increase in activity at 15.5°C. There was partial paralysis of the legs at 24.9°C, quiescense at 26.4°C and final heat prostration at 27.8°C. This interesting work on the temperature relationships of this insect clearly demonstrates that a temperature which is compatible for most insects will produce death in the grylloblattid. It also shows the grylloblattid is very active at temperatures at which most insects would go into dormancy. Active grylloblattids are often collected from beneath objects which are frozen to the earth.

Indications from Walker's laboratory in Toronto are that the life cycle of the insect takes about 10 years. It is unusual in its development in that there is a pre-adult stage, the sub-imago. This is very unusual, the Ephemeridae or May-flies being the only other insects with such a stage. During the sub-imago the insect is white, lives in the ground and does not appear at the surface. After the last moult it assumes a honey yellow color. This information has not been published to date but was brought out in a conversation between Dr. H. B. Mills and Dr. E. M. Walker.

An insect which requires such a low temperature scale might possibly be the source of a psychrophilic bacterial flora. With this in mind the investigation of the flora of the alimentary tract was begun.

Literature on the subject of insect-bacterial relationships is surprisingly limited. It would be supposed that, from the possibilities of biological control of insects, more investigations in this field would have been made; also that with regard to the importance of insects in the transmission of disease comparatively few species have been examined as to their bacterial flora. Steinhaus (1940) has recently surveyed the field and presents the best compilation of previous work done on this subject with regard to all phases of the biologic relationships existing between insects and the bacteria they harbor.

Leydig (1857) noted the presence of organisms in the caecal appendages of a pentatomid. Forbes in 1882 discovered numbers of bacteria in the caecae of certain Heteroptera.

Glasgow (1914) while working on the flora of the gastric caecae developed an aseptic method for removing the caecae as follows. "The insect is first lightly chloroformed to prevent struggling, the wings are clipped off near the base and the whole body moistened with alcohol to remove the film of air and allow the penetration of the bichloride solution which was usually used in the proportion of 1-500. The mercuric chloride solution is best applied with a bit of absorbent cotton held in a pair of old forceps. In this way the entire body of the insect can be thoroughly scrubbed with the disinfectant, so that any folds, such as those between the body segments, will certainly be moistened. After the bichloride solution has completely dried, which may be very well hastened by passing the insect back and forth before a Bunsen flame, the flat edges of the abdomen are clipped off, from near the posterior end up to the thorax, with a pair of fine scissors which have been previously flamed. The top of the abdomen immediately back of the thorax may be cut across with sterile scissors and the resulting flap formed of the entire dorsal wall of the abdomen may then be lifted back with a pair of flamed forceps, leaving the abdominal viscera exposed."

Glasgow was able to get good bacterial smears from the caecae of certain species of Heteroptera, but could not obtain cultures on ordinary media. With other species he obtained luxuriant growth in nutrient broth. A squash vine infusion broth composed of nutrient broth and a decoction from 150 grams of squash vine per liter was successfully used in cultivating caecal organisms from the squash bug Anasa tristis. Glasgow found that the caecae of the Hemiptera of any one definite species invariably contained a pure culture of but one species of bacterium. The bacteria from different hosts varied greatly, from small coccus like organisms to large spiral forms. However, "in whatever form they occur they are morphologically characteristic for the particular species harboring them." Glasgow believed that these normal bacteria appear not only to inhibit the development of foreign bacteria but to exclude them altogether. This is a very interesting observation.

Pierce (1921) gives a summary of the records which have been made of the organisms isolated from cockroaches. Fifteen species of bacteria are listed as being either isolated from the feces of this insect, or the alimentary tract. Pierce also lists 53 species of bacteria that have been