THE USE OF GERM FREE GUINEA PIGS IN BACTERIOLOGY

1. PRELIMINARY REPORT CONCERNED ESPECIALLY WITH TECHNIQUE

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The problem of raising animals in a germ free condition has intrigued many bacteriologists philosophically but has not been followed by much experimental investigation. Consequently there is a dearth of evidence in the literature concerning this problem. No doubt the popular aspects with which the problem has always been presented together with the technical difficulties involved has resulted in the casual interest with which it has been treated. The available evidence shows much contradiction and the problem is today far from being practically settled in regards to many animal forms. With guinea pigs, however, evidence appears to support the view that the young may be obtained and raised in a germ free state.

The present series of experiments on germ free animals were initiated by a desire to obtain living culture media, free from contaminating influences and as near to natural conditions as possible for use in investigations concerning microbial heredity and change. When used for investigations of this nature the problem, in this instance, assumes a practical aspect. Such animals in a germ free state present ideal testing grounds for this type of work and also for investigations concerning bacterial associations. For example it is possible to add one bacterial cell or a certain cell from a particular strain to a germ free animal and watch the changes which occur in the bacteria. It is also possible to build up an artificial intestinal flora by adding the constitutent organisms one by one. Germ free animals also offer a rare opportunity to study phage and filterable stage developments directly.

Guinea pigs were chosen as experimental animals because they are easy to infect, easy to work with, and are born, normally in a more or less mature condition. The young pigs are obtained by caesarian section and are raised in a special cage. Such apparatus while necessarily complex must allow easy access to the animal without exposing it to the dangers of infection. Many physiological factors enter into the operation and raising of germ free animals. A consideration of these factors will be taken up in a later publication. For the present it must suffice to say that the adult female must be within a few days of term and should be anesthesized with a compound similar to sodium amytal. If more than one foetus is present it is seldom possible to obtain more than the first one in a satisfactory state for the experiment. The young pig once it is placed within the feeding cage must be fed at short intervals,

day and night, until it can not only shift for itself in the matter of food, but can digest what it eats. The question of diet has presented many difficulties especially during the early life of the pig. The difficulty of obtaining suitable food after it has been sterilized in the autoclave is still a matter of experiment.

Mortality is high for the first few days of life and its chief contributing causes might be listed as, damp air, irregularity in the feeding schedule, temperature changes, shock following the operation, diet and causes which cannot be exactly determined.

In my experiments with germ free animals the usual controls were set up. Air supply, food, nasal washings, surface of the skin and the water used in the experiments were brought into contact with different culture media. The usual microscopic tests on the blood and exudates of the animal were made after its death. It might be remarked, however, that no attempt has been made up to the present to test for filterable vira or other submicroscopic principles.

Up until the present time I have been concerned with the design of the apparatus and technique used with the different steps in obtaining germ free guinea pigs, in an attempt to simplify and develop a more practical technique. The apparatus used for this purpose may be seen in my laboratories. A full description of the apparatus together with the methods employed in this work will be submitted to the editors of the *Journal of Bacteriology* at a later date. In working with the apparatus it has been necessary to operate a certain number of animals for test purposes. The data and results concerned with these operations might be summarized as follows:

Eighteen female guinea pigs which might be counted as part of the experiment reported at this time have been operated during the course of two years. These animals were delivered of 43 young, 24 of which were delivered alive. The high mortality in this instance might be accounted for by technical factors such as handling, use of improper anesthetics, improper care of the delivered animal, etc. Of the 24 pigs delivered alive only six animals were delivered germ free into the incubator. Three of these animals developed bacterial infections resulting from contamination of food, handling or faulty apparatus. The three pigs which were shown germ free, within the limitations of the aforementioned controls gave the following results: one lived four days and was killed after it became much weakened from faulty diet, one pig lived five days but died from unknown causes (probably improper feeding intervals or improper food) and one lived eight days and was then killed in an apparently normal condition.

Operating for germ free animals and their further culture presents a good many technical difficulties. Good assistants, and experience in details of the operation are necessary to the work. Animals in a germ free state delivered with the present technique require the closest attention as to feeding details. The physical strain placed on the operator in nursing these animals is great. Since the work requires night and day attention for the first few days and more or less constant attention after this period and is tedious it is seldom possible to continue the experiment for a long period of time unless assistance is at hand. No

doubt after the animals have been carried over the most difficult period and are being fed at longer intervals they will require less attention if the apparatus is properly constructed. In the final analysis the experiment is largely a matter of mechanics.

These difficulties are mentioned not to dicourage but to explain the abberrant results reported on the first series of experiments. More recent experiments gave better results. Certainly the value of such animals in experimental investigations concerning bacteriophage, filtrable vira and life cycles warrant further investigation along these lines.

SUMMARY

It is my purpose in this paper to point out the practical possibilities of using germ free animals in bacteriological investigation especially when the research concerns life cycles or change of bacteria. Experiments show that guinea pigs can be obtained and raised in a germ free condition and put to practical use only when the technique and apparatus is especially adapted to that end. It is believed that the apparatus shown in my laboratories simplifies the technique and adapts it to this practical end.

THE MECHANIZATION OF CERTAIN BACTERIOLOGICAL PROCEDURES

A CONSIDERATION OF THE FACTORS INVOLVED IN MECHANIZING SINGLE CELL TECHNIQUE AND THE PLATING METHOD OF COUNTING VIABLE BACTERIAL CELLS

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This paper is concerned with a discussion of the mechanization of two important bacteriological procedures, (1) single cell isolation and culture, (2) the plating out of bacterial cells in a nutrient agar for the purpose of estimating the viable cells.

Single cell methods are important in selecting pure strains of bacteria or certain organisms from a pure strain. Its practical applications are concerned with the preparation of antigens, the diagnosis of certain diseases and its use in research bacteriology.

Plating technique as referred to in this paper is used to count viable cells in samples such as milk or broth cultures. Its practical application concerns its use in the milk industries or in problems such as the study of growth curves where a great many counts must be made.

Both procedures as they stand at the present time allow too great a personal factor to be consistent. Furthermore they do not conform close enough to mechanistic principles to permit their easiest manipulation in routine tasks. With these ideas in mind an effort has been made