August 26, 1946.

Dear Dr. Edwards:

It was most gratifying to hear from you, and to find your interest in our work. It is particularly fortunate, I think, that we do differ in our formulations of the Salmonella story, realizing that I have had to approach the matter on an entirely a priori basis with some genetic ideas transported from other organisms, while you have and all the practical and experimental contact with these organisms.

The cultures and serums which you sent have been received in good condition, and we are set to go to work with them immediately. However, a new tack has suggested itself which modifies somewhat the approach I suggested previously. There seems to be already available in many Salmonella types a diversity of nutritional requirements, which should be as usable as induced nutritional mutants in detecting gene recombinations. Therefore, instead of selecting, at this stage of the game a particular antigenic type, a large series of Salmonellas will be worked over for their nutritional requirements, and those selected which will be on that basis most convenient. I have been able to obtain a few varieties already from the Columbia and Yale Laboratories, and have enlisted Dr. Wheeler's cooperation in extending that list.

There seems to be a most striking analogy between phase variation in Salmonella and some features in the inheritance of mating type in Paramecium bursaria as described by Jennings (Proc. Amer. Philos. Soc., 85:25, 1941), in particular the phenomenon of 'self-differentiation'. Jennings finds that a clone of a given mating type (of which there are four alternatives) may give rise to organisms of a different type; dif-

ferent clones of a given mating type characteristically 'self-differentiate' to a given alternative, which may be different for different clones. The clone obtained in this way may in turn 'self-differentiate', always to the original type. Although Paramecium is a sexual organism, the genetic basis of this phenomenon is still quite obscure, and hybridization experiments so far have shed no great light on the matter. I do not know whether any attempt has been made to characterize mating types serologically.

It will be some time before the survey of nutritional requirements in Salmonella will be completed, but we shall, of course, keep you informed in detail of our progress. While Dr. Wheeler's collection should be adequate, we would appreciate the opportunity to ascertain the nutritional requirements of any strains in which you may be particular interested.

Re-reading my original letter to you, there seem to be some considerations that I did not express quite clearly. In order that there may be no misunderstanding, the situation should be reviewed:

a) The E. coli work demonstrated that there are genes in bacteria which determine such characters as nutritional requirements, and phage susceptibility.

b) In E. coli, different substrains can be hybridized giving rise to new types, characterizable as recombinations of the different characters as the parents.

We have no evidence as yet as to the inheritance of antigens, nor as to the occurrence of gene recombination in, or between Salmonella strains. In E. coli, the recombination of nutritional requirements can be used as a basis for selecting out of a large number of cells those few in which recombination has occurred. In such cells, the segregation of other genes can be determined.

In Salmonella, recombination of nutritional requirements must first be determined. If this occurs, we are in a position to study the inheritance of other characters (such as antigens).

Only if a system of inheritance of antigens can be formulated on the basis of 'crosses' can the problem of phase variation be studied. It probably will be best to use a form which is normally almost entirely monophasic, but in which the alternative phase can be detected and selected for by well-known methods.

Parenthetically, the expression 'Induced Variation' has a rather different connotation in genetics from that in bacteriology. It is taken to imply a direct action of the inducing agent on the hereditary materials of the organism. Unless there is direct evidence to the contrary, cases of apparent induced variation are formulated as instances of <u>selection</u> in favor of a small proportion of spontaneously occurring variants. Your own work seems to bear this out in Salmonella In the bacteria, only the pneumococcus transformations have been accepted by most geneticists as bona fide examples of 'Induced Varaition'. There may of course be others.

It would be most convenient to have a set of specific Salmonella phages. Can you suggest where they might be obtained, and what references there may be in the literature?

Finally, I hope that I shall have an opportunity to visit your laboratory sometime in the next year, but there is nothing definite in sight as yet.

Very sincerely yours,

Joshua Lederberg.