PUBLIC HEALTH LABORATORY SERVICE
(Directed by the Medical Research Council for the Ministry of Health)

Central Enteric Reference Laboratory & Bureau

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~~CENTRAL PUBLIC HEALTH LABORATORY~~,
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LONDON, N.W.9.

10th February, 1953.

Dear Dr. Lederberg,

I must apologize for not having written before to thank you for your letters of the 5th and 23rd January and the attached Summary of the paper by Mrs. Lederberg and yourself on "Genetic Studies of Lysogenicity in Escherichia coli". The impressive collection of reprints has also arrived safely. I am rather slow in writing letters and, in addition, I waited a few days to have some of the old cultures ready for despatch before writing to you.

Naturally, I studied the contents of your two letters with great interest and very much enjoyed some of your remarks. I must admit that I do not feel competent to follow you into the vast realm of genetics, but somehow I feel entitled to dislike and distrust Luria's "parasitism at a genetic level". To my simple mind it appears that what happens at this level are reactions that will be clarified one day by enzymologists rather than by virologists of the type I knew in the older generation. I know I shall not live long enough to witness this development. On the other hand, your own work and that of your school is advancing so rapidly that I seem to have a good chance of reading one day that the parasite theory of bacteriophage is untenable. I do believe that the only basis on which bacteriophages may be "specific self-reproducing units which form part of the genetic make-up of the bacterium", is the one assuming that they are "endogenous products of the bacterial cell." I do not believe that "the question of endogenous vs. exogenous devolves mostly on a question of how long ago" (see page 2 of your letter), or that there is any "further evolution of phage into a more independent organism" (see page 3 of your letter). You will appreciate, therefore, that I was delighted to read the last paragraph of your letter of the 5th January, where you discussed the results of the recent work on phage lambda by Mrs. Lederberg and yourself.

I feel on more familiar ground on turning to the various points relating to serology and antigenic analysis, which you have raised in your letters.

1). Reversion from O variant to O + H variant in strain O 901. - It was certainly a surprise to me to learn that the cultures of strain O 901 which you received from Kauffmann and Boulgakov readily revert to the O + H form. It is true that in my own laboratory O variants of any organism handled, are, as a rule, maintained on dry agar slopes or plates, free from water of condensation, to avoid encouragement of development of H antigen. However, since this strain has been first recommended for use in the "Qualitative Serum Diagnosis of Enteric Fevers" (Lancet, 1930, 1, 505), it has been distributed to many workers throughout the world by the National Collection of Type Cultures or by myself, and I have not heard before of its reverting to the parent form H 901.

I enclose a reprint of a paper on the "Standardization of Diagnostic Agglutination Tests", published in the Bull.World Hlth Org., 1950, 2, page 643, which I had not sent you before. On page 645, line 1, you will find the following statement:

"(2) in most laboratories throughout the world the suspensions for the estimation of TO and BO agglutinins are now made from cultures of the permanent O variants of the two special strains recommended for the purpose by Felix (1930)."

In this country the suspensions distributed by the Oxford Standards

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Laboratory were made for more than ten years from broth cultures of these two strains; nevertheless, reversion to the O + H variant has not been observed (see also Felix & Gardner, Bull.Hlth. Org. L.o.N., 1937, 6, on page 234).

I sent you today by ordinary airmail a parcel containing duplicate Lemco stab cultures of the two strains H 901 and O 901. The cultures were specially prepared from the oldest stock cultures available and are labelled as follows:

O 901 No.1 derived from old Lemco stab culture dated 18.9.1935
O 901 No.2 derived from old Lemco stab culture dated 1.2.1938
H 901 No.1 derived from dried culture dated 5.6.1936
H 901 No.2 derived from old Lemco stab culture dated 24.2.1937

I took the precaution of going back to the oldest available stock cultures because you are employing these strains in experiments with various bacteriophages, and I wanted to make sure that the cultures have not been accidentally exposed to any bacteriophages in my laboratory, where bacteriophage work did not start until 1940. I shall be very interested to hear whether these two cultures of O 901 can readily be made to revert to the O + H variant.

2). Anti-O phages - I am afraid you will find the information I am going to give you on this subject rather disappointing. The source of this disappointment is Kauffmann's wrong teaching. At first he neglected the existence of the numerous partial O antigens in the complex O antigens of the various Salmonellae; subsequently, he and his followers attempted to carry the differentiation of the various O-antigen complexes into minute details, without realizing or admitting the limitations of this technique. Consequently, the information given in the Kauffmann-White scheme under the heading "O antigen" has always been misleading. In the early years it was an over-simplification; later on the tables conveyed the impression of a degree of accuracy which, in fact, they did not possess. Undue reliance on the slide-agglutination technique was one of the reasons for those mistakes.

You may have seen my recent papers in the December number of the Journal of Hygiene, 1952, 50, pages 515-579. I may refer you to pages 525-526, and again to page 546, where the question of the complexity of the Salmonella O antigens is briefly discussed. The reprints of the four papers have just arrived and I am posting them today under separate cover by ordinary mail.

Table 1 of the Oxford Symposium, to which you referred in your letter of the 23rd January, was primarily intended to show in a general way the remarkable parallelism between phage action and antigenic structure. At the same time the table showed that the lytic action of Anti-Vi and Anti-H phages is "relatively specific", whereas those of anti-O and anti-R phages are "widely over-lapping". The anti-O phages referred to in the table are exemplified by the three phages (Numbers 1, 2 and 3) I sent you recently, whose lytic spectrum extends over the great majority of Salmonella species belonging to all the different O groups in the Kauffmann-White scheme. I thought that the "widely over-lapping" i.e. relatively non-specific action of these phages was adequately indicated in the table.

In the original paper (Brit.med.J., 1943, 2, 127) these anti-O phages were described as follows: "It was soon found that most of the phages obtained from these sources were anti-O phages that acted on paratyphoid B bacilli as well as on Bact.typhi-murium bacilli and many other members of the Salmonella group. Even minor O-antigenic components that are common to the various Salmonella species, though they are not listed in the Kauffmann-White diagnostic scheme, provide an adequate point of attack for anti-O bacteriophages." (See page 2, lines 9-15 in the reprint of the 1943 paper which I sent you last year). On the same page, line 32, you will find that these phages were referred to as "the non-specific O phages" as contrasted with "the specific Vi phages".

It is obvious from these quotations that I never expected to be able to identify, by serological methods, a presumed common receptor of these O phages.

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I know, of course, that other O phages have a much more restricted lytic spectrum. Nevertheless, I would be much surprised if it were possible, even in those instances, to associate the phage receptor with a serologically identifiable O-antigen fraction.

Vi-negative

Broadly speaking, I would define as O phage any phage acting on a/ bacterial cell that still contains some "smooth" O antigen, demonstrable by serological methods. The quantity of antigen present may be very small indeed, and may not be demonstrable by any test in vitro, only by immunization of rabbits. A partially rough variant, which is salt-agglutinable and shows other criteria of "roughness", may nevertheless contain a considerable amount of "smooth" O antigen.

In this connection I would like to refer you to the paper published with Anderson in the Journal of Hygiene, 1951, 49, page 349, dealing with a strain of Salm.typhi that contained the TVi antigen in such a small quantity that the cultures might easily be passed as Vi-negative forms, when tested by the customary routine technique. These cultures were, nevertheless, fully susceptible to Vi phages.

3). Anti-R phages. - This may be the place to mention anti-R phages. You wrote in your letter of January 23rd: "I wonder whether a second phage, possibly rough-specific, may not be responsible", in order to explain Boyd's seemingly contradictory finding that "A1" lyses S.bovis-morbificans. In the light of what I said in the preceding paragraphs the assumption of a second phage does not appear to be necessary. I should have mentioned before that each of the three O phages I sent you had, of course, been purified by repeated single-plaque isolation, so that the question of a second phage could not arise.

The anti-R phage listed in Table 1 of the Oxford Symposium lyses "rough" variants of all the different Salmonella species that have been tested against it, but does not act on the corresponding "smooth" variants. This is in good agreement with Bruce White's finding about the serological, and presumably chemical, identity of the R antigen throughout the whole Salmonella group. To be exact, I ought to mention that in all the bacteriophage tests I have been discussing only lysis has been considered, not absorption of the phages.

4.) Anti-H phage. - You may be right in what you wrote about insufficient study of this phage. I have had no personal experience of it and included this phage in Table 1 on the authority of the authors quoted in the text, most of whom I had good reason for accepting as competent workers. After the death of my old friend Schiff I tried to obtain the anti-H phage from New York, but it was no longer available.

A few weeks ago Dr. Stocker sent me Boulgakov's phages (Phage VIII-113 propagated on 377 and Phage VIII-113 propagated on 372) the first of which he had received from you. I have not used these phage preparations so far. Sertic left the Paris laboratory more than ten years ago, and since it is a commercial laboratory I would be reluctant in accepting Boulgakov's phage as an authentic derivative of the phage originally described by Sertic and Boulgakov (1936) and later employed by Schiff and his co-workers.

5). Booy and Wolff's paper. - I had some difficulty in tracing this paper, which had escaped my notice. However, a few days ago Professor Dinger, the Director of the Institute at which Booy and Wolff are working, wrote to me, and this gave me an opportunity of telling him my opinion of the paper. Instead of re-writing it I enclose a copy of the letter. Naturally, this is intended exclusively for your personal information.

6). Professor Créze has not written to me and I have not read anything published by him. I am not quite clear about what you meant by "recommending his address" to me. So far, I do not know Professor Créze's address.

7). Although this letter has now become very long I would like to add one more remark. You wrote that the O phages I sent you "will not be directly usable in transduction experiments as they leave very few survivors from most of the Salmonella cultures exposed to them". In experiments with these phages some years ago I never succeeded in sterilizing cultures of a number of different strains of Salm.typhi. Neither an O phage nor a Vi phage could

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accomplish that, but a mixture of the two produced complete sterilization. This is mentioned briefly in the Proceedings of the 4th International Congress for Microbiology, Copenhagen, 1947, on page 363. On the basis of this experience I assumed that most of the Salmonella cultures exposed to these O phages would leave surviving resistant mutants in numbers suitable for your experiments.

I hope this time the small parcel will be treated with less suspicion by your customs authorities. I would like to add that, for some obscure reason, these Lemco cultures survive better at room-temperature than at 2° to 4°C.

Yours sincerely,

A. Felix

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