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Dr. Joseph Lein
Bristol Laboratories Inc.
Syracuse 1, New York

Dear Joe:

Your short record inquiring about resistance of protoplast just received and I will answer this first as it is fresher in my mind.

I am a little surprised that you found no difference whatsoever in your procedure, but the protocol is actually quite different from the one that we used. You are testing a differential effect of streptomycin on protoplasts in the course of their reversion from the protoplast to the raw state. Soon after you plate the protoplasts onto a sucrose medium free of penicillin, they will begin to revert, and, unless they have been irreversibly damaged in the meantime by their exposure to streptomycin, they can be expected to survive. Our experiments were done in a slightly different fashion, mainly to test the effect of the antibiotic on the capacity to form L colonies in deep sucrose penicillin agar, that is to say, the protoplasts were allowed to develop into L colonies in the continuous presence of penicillin. Under these circumstances, we found whether we had a sensitive or resistant clone that the streptomycin would greatly impair the yield of L colonies. This raised the question, of course, whether this was a unique effect of streptomycin on protoplasts and their colonial forms or whether there was some other mode of interaction between penicillin and streptomycin. In order to do that, we performed the experiment in a slightly different fashion, namely, using a DAP-less mutant and permitting it to form protoplasts and L colonies by deprivation of DAP. We obtained exactly the same results, that is to say, a streptomycin resistant DAP-less mutant was sensitive to streptomycin in so far as L colony formation was inhibited by relatively low concentrations of streptomycin even for a resistant mutant. We did get some evidence of interaction of low concentrations of penicillin and streptomycin and it is for that reason that I was surprised that you got no effect whatsoever. However, you mentioned that you had 90% conversion of cells to protoplasts. This is to my mind a rather low figure and suggest that you did not have complete conversion at the time that you did your experiment. I suspect that if you tested your suspension at a point where the osmotically fragile fraction was 99% or higher that you would have obtained a differential even under the conditions of your experiment. So it would look to me as if you had an incompletely protoplast preparation which was competent to revert fairly rapidly when plated, and that under these conditions, it was not enough irreversible damage by streptomycin or the other antibiotic to make a difference. I hope this will clear up the situation.

As you may have noticed in Nature and elsewhere, there is considerable controversy now going about whether we should call these elements protoplasts. This is very much a somatic question that I have no particular interest in getting into and if there is a universal consensus that the E. coli bodies whether produced by lysozyme or penicillin should be called something else like "speroplasts" I would be perfectly willing to go along. The main point is that the cell wall of Gram-negative bacteria is very much more complex than that of Gram-positives and that penicillin is hitting some specific

component of the wall rather than the wall as a whole which seems to be quite self-evident. We have not, I am sorry to say, been doing very much more work along these lines for a good many months as we have been concentrating on problems of sexuality and transduction.

The next bit of news that I would like to give you, Joe, is that we have accepted a proposition from Stanford University to found a department of genetics in the Medical School at Stanford. We will not move to the Stanford campus until next July. Our present plan will let us take a three-months research trip to Italy to work with Cavalle just prior to our final move. I hope to have a chance to talk with you about the possibility of some collaborative work involving Dr. Cavalle or some of his associates as I think that he is part of a brilliant group that could contribute a great deal.

Now to take up some of the questions on the record that we received about a week ago. Well, as a matter of fact, Joe, a couple of days have passed and this is now July 23. I will definitely plan to visit you this fall to go over the accumulated questions in detail. I hope you have no fear that my move out to Stanford is going to impede in any way the continuation of our very gratifying dealings. I don't think that it is going to turn out that Syracuse will be any further by air from Madison than it is from San Francisco, and especially with the establishment of jet service late this fall, it should certainly prove to be true that the through connections from San Francisco may even give an advantage in time as compared to Madison. I hope, too, that our being out there will give you some excuse to revisit the west coast for which I know you have some predilections, but that will be some time off, of course.

Now, as concerns the conditional antibiotics which are effective only in minimal medium, it seems to me that the only likely interpretation is that the complete media have metabolites which antagonize the action of the antibiotics. Since those which have tumor activity are most likely to be related to nucleic acid, it seems to me, you might be able to test out this notion by comparing the effects of these antibiotics on some simple mixtures including synthetic amino acid mixtures or casein hydrolysate together with the complete medium. It seems to me that we have been over this before and there must be something wrong with my suggestion or the problem would not still be current. In fact, didn't you tell me at one point that the reversing activity for one of your antibiotics was either leucine or isoleucine? But, never mind, even so, I think you are going to find that it is some of the non-amino acid components of your complete media that are at fault, and this should not be too difficult to run down and will of course give an important clue on the mode of action. I still haven't got the foggiest idea, really, why E. coli K12 should be so uniquely sensitive as you described in an earlier communication.

On the question of scaling up production, I am very glad to hear that this is no longer a serius problem, but I wonder if that is going to be entirely true in every respect. Surely there are times when you are discouraged from making further investigations by relatively poor yields on your medium scale operation. In any case, I would think one would want to know what the factors are that are responsible for the difference, but if they haven't been worked out until

this point, I don't believe that my particular remarks would warrant a specific search for them.

Your comments on reasons not to go into phage or the limitations of phage therapy seem to me very well taken, and the idea of going after a phage system where antibiotics have not worked is the soundest point of view that one should take. I don't know very much about the mycobacterium phages, probably no more than you would by looking at one or two issues of the Journal of Bacteriology where they have been reported. I think there is also a paper in the Canadian Journal of Microbiology, and if you remind me, I will look into it this fall. As to pseudomonas phages, it happens that I know a fellow who has been working very actively with them, and this is Bruce Holloway, whom you may possibly have met at some time. At any rate he did his post-doctoral work at Cal. Tech. on Neurospora heteracarions and went from there back to his home in Melborne, and he was one of the fellows I interacted with in our trip down there last fall. He has made a fairly substantial collection of pseudomonas phages in hopes of using them for genetical work, and I would think if you were seriously interested in this, either you or I could approach him and ask to get them. In fact, it is just conceivable that he has sent some in some earlier packages, but I will look a little further into it. He is coming to this country this fall for a tour of congresses and laboratories and will be at the Montreal Congress. If you happen to be going to the Genetics Congress in Montreal, you might have a chance to meet him. His name again is Bruce Holloway and he is, together with a chap called Loutit in New Zealand, about the only one working on pseudomonas genetics at the present time. On the nature of the bactericidal element in phage, I think that Herriott's papers, which came out last year in the Journal of General Physiology, are the last word on the matter. I was perhaps a little bit previous in talking about a protein. Of course, I'm speaking about phage ghosts which have lytic activity There are still some obscurities connected with it.

I don't think that nucleic acids are going to be very useful to you in the present state of the art, although they may ultimately get to be. In the tobacco mosaic virus story, it has not been possible to recombine virus nucleic acid with miscellaneous proteins, although it was possible to interchange protein from one virus strain with that of another. We may eventually learn the trick that will be needed to make this approach useful in bacteria phage, but at the present time it seems to me that what we might really be interested in is the specific protein of lytic phages since this may be the pure element involved in bactericide. Even under the best conditions at the present time the order of infectivity of nucleic acids for bacteria is extremely low and I think we will have to wait for further technical developments to use this approach. As a matter of fact, I should think to ask Esther about that question of pseudomonas phages because she has had a little bit of experience in playing around with it. At the moment, we are busier thinking about packing than anything else, and I would like to forgo this until we get back. The transcript of this record will be a sufficient reminder.

On the question of substitutional chemotherapy, I'm very happy, but will not say surprised, that you are getting what may turn into some promising leads. After all, at least to the stage of getting growth inhibitors the approach was bound to work, and now the question is whether this can be put to any practical use. I would like to stress that I am rather doubtful that the kind of methods that you are playing with now for searching for new inhibitors

are likely to be the ones used in their eventual production. I view this as a screening program on the largest possible scale for the largest variety of antimetabolites, but I would be hopeful that you are going to run into compounds which can be prepared by fairly straight forward organic chemical procedures once they are identified. But of course you should get a complete hodgepodge of all kinds of things and that is really what we are hoping for. I don't think that the details of the first method of approach are really terribly important. One has to grope around in the dark for quite a while and get his bearings on a clinical basis before one can have any particular judgment about what the best method of approach is going to be. So more power to you for sure.

As to whether to use more purified precursors or the whole cells in the first trials, I don't think there is really very much of a choice. The ultimate value of using whole cells is that you will introduce compounds which are, on the one hand, not especially well known to science at the present time, and on the other, these may very well be just those metabolites which are unique to the bacterial target and may be absent from the host. On the other hand, the sorts of compounds that you are throwing in, carbohydrates, amino acids, etc., are going to be just as prevalent in the host as in the parasites and there will be at least no rational basis for specific inhibition of bacteria with materials obtained on this basis. That is by no means to say that they are not going to turn out that way.

I had not yet seen that paper on actinophage that you refer to, but will look it up when I get back. Galen Bradley has been working on that type of system for some time and he is pretty well convinced that most of the effects reported in literature so far are entirely due to selection of spontaneous resistant mutants, but I had better look at the article before I say very much more about it. I think if you decided some point to get very expansive about getting down to brass tacks in actinomycete genetics, you might want to talk to Galen and see if he has anything tangible that might be useful for you. At the very least why don't you ask him to come out and give you a seminar sometime so as to wrap up his recent work. He has been working very hard during the last couple of years and I think has at least as good a perspective as anybody. We are rather hoepful that Sermonte who has been working in Rome and doing very splendid work, indeed, will also be coming to the States this fall, but I'm a little confused as to whether he will be working with Bonner or with Bradley or both. He is another fellow I think you want to get to meet.

Now, as to the papers from the Bristol Laboratories, I honestly think, Joe, that there really should be no question about it, and I would welcome, in fact, being on your total mailing list, and I mean total. I think the chances that any single one of these is going to be particularly pertinent to my work may be small, but you never know, and I would be very pleased to have this additional information bearing on your overall operations. I'm not going to have a chance to look at them, of course, until I get back this fall.

If I didn't mention it in the first part of the record, our itinerary is pretty well fixed now and we will be abroad until about the 5th of September, then we're flying from London directly to San Francisco and will be out at Stanford for a few days until the 10th of September before we finally return back to Madison. I hope this is going to be my last very extensive traveling for a little while to come, but I'm going to exclude from that remark the trip that I am definitely planning to make through Syracuse.

All best wishes.

Yours,

Joshua

JL:ac