MEMORANDUM

BRISTOL LABORATORIES

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lederberg

DATE November 7, 1955

TO J. Lein

SUBJECT New Antibiotics Screening Program - Consultantship Arrangement

C.C.

Dear Joe:

We gather you have had some interesting travel lately. I've been running around a bit myself but find the experience more exhausting than stimulating. I am going to keep in mind the possibility of making a visit to you before the end of the year but I can't say just yet when it will be possible. One of the complications is that I am teaching and have classes on Tuesdays and Thursdays. This makes it a bit awkward for a visit which requires the best part of a day to get there and a similar time to get back. I would be curious to know how happy you and your colleagues would be about a visit on a Saturday or on the Firday of the Thanksgiving weekend or similar dates. During the Christmas recess or after the present semester this problem won't be nearly so urgent.

To take up the subjects in order, of course I have seen the properdin work in the newspapers but I hardly know anything about it but I will keep my eyes open and make no comment until I have familiarized myself a little better with that situation.

I am happy to note that you are getting somewhere with the induction system but I have the feeling that it has not yet been perfected to the point where it would be really worthwhile to use in its present form. While, as you point out in your discussion, you do get a 100-fold increase in the amount of phage over a period of 5 hours with one gamma azaserine, this really should be compared to the amount of phage that you get in similar cultures without azaserine and the comparisons that I see here would be the order to 10^5 plaques/ml in M-9 alone as compared to perhaps twenty times that much in the presence of azaserine. Considering that azaserine has already been purified and demonstrated elsewhere to be a very efficient producing agent this still does not seem to me to be a very satisfactory differential.

Looking over the protocols I would have the following suggestions to make by way of sharpening up the system. First of all, it is quite true that azaserine is of course antagonized by other substances in nutrient broth. I understand that the aromatic amino acids are especially efficient in this respect. On the other hand, I do not think that you can get anywhere near optimal induction in synthetic media, especially without aeration. I would have the following concrete suggestions to make by way of modifying the technique. First of all, either to improve the conditions of growth in the synthetic medium specifically with the help of aeration, preferably on a rotator or shaker or something of that sort, or else to conduct the induction in a complex medium. In my own hands M-9 is not as satisfactory a medium for the growth of E. coli as is the Davis minimal medium which is described in some of my papers and also in Bernie Davis' but we have found that under any circumstances or any media that aeration is quite necessary in order to achieve maximum yields of phage. My second point would be that it would probably be more effective in terms of induction if you could treat the bacteria for a relatively limited period of time, say one hour or two hours, in the presence of an inducing agent and then either dilute or, in place of that, wash the cells so as to replace the medium with one that is optimal for growth.

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In my own opinion the best regime would be something as follows: Use 8 hour or 10 hour inocula from a complex medium, nutrient broth, or, Penassay medium is one which we have used very effectively. Wash the cells and resuspend them at a density of about 10^8 cells per ml in a synthetic medium in the presence of whatever compounds you are trying to test the induction powers of. Incubate with aeration during this interval for about 2 hours. Then either dilute or wash the cells and replace them with an aerated Penassay medium. Another 2 hours should be quite sufficient for the completion of release of any phage that is going to be released by the induced cells.

I think with such a technique you should get, with an agent like asaserine, levels of induced phage at least 100 or perhaps 10,000 times higher than you are now recording. This will of course involve using somewhat denser suspensions of bacteria during the treatment. I am also concerned about the rather high basal level of the phage that you are reporting and I suspect that the difficulty here is that you are not using quite enough streptomycin in the indicator plates. I would certainly try at least 50 and preferably 100 or even higher concentrations of streptomycin, that is, in gamma per ml, in making the assays. I suspect that at 10 gamma/ml the lysogenic bacteria continue to produce a small amount of phage in the assay plates but high concentrations of streptomycin should stop them much more abruptly. With washed cells you should get a count of plaque formers in your untreated suspension which is considerably less than one per 1000 of the colony formers--considerably less. I would imagine that the results you are getting now would be pretty much the same as if you were not adding streptomycin to the indicator medium.

I don't believe that we will have any further or additional use of a W14-85Sr strain at the present time but thank you anyhow, Joe. I do think, however, that you might find it advantageous to look into the use of a few other strains of sensitive bacteria, for example, strain C, which I believe we have sent you before.

The considerable variability in plaque size that you mentioned is not uncharacteristic of random platings. It's one of the serious headaches of this particular system and I don't know too well how you are going to get around it. I suspect that the plaque variation comes about partly from the fact that through a plating, both free phage particles and infected centers which are shortly about them in these very large numbers of phage particles, the variation may disappear or at least be greatly reduced with the regime that I have mentioned before or with the incorporation of more streptomycin into the assay plates. Something else might conceivably be happening, viz., that your stock culture has picked up a certain amount of a mutant to phage which we call lambda 2 which is capable of growing lithely on the K-12 strain. You can rather easily check to see if any of the rather clear plaques that you have in your assay plates are this mutant by simply determining whether they will lyse the K-12 strain itself. If this is the trouble, or in fact even if it isn't, there might be some advantage in using as a test culture a mutant of K-12 which is resistant to lambda 2 which mutation did not interfere at all with its ability to release lambda under conditions of induction and we will send this on to you in awhile. This is the so-called lambda 2^r or LP_2^r mutation. If I may summarize the following are points which I think will contribute to giving you a much sharper assay than you have now:

1. Conduct the treatment of the bacteria in a synthetic medium but subsequent to a very brief treatment they should be allowed to develop and grow the phage in a complex medium under conditions of aeration.

2. Use considerably higher levels of streptomycin in the assay plates.

3. See whether the extreme variation in plaque morphology is due to the presence of a lytic phage which will attack the K-12 strain. If this is the case, it may partly account for the high levels of control phage that you are running into.

In my own opinion it would not be profitable to proceed with the testing of new agents until you have a system which is rather more sensitive to an agent like azaserine.

To turn to some other issues, I am very pleased to learn that you will have some continuing interest at all in the problem that is related to the use of the Serratia strain that we sent you. When you said you would feed it to see if it becomes established, I hope you mean to give the red bacterium some encouragement. I would very much doubt that it would displace a pre-existant enteric flora in the mouse. I would have some hope that it might do so under conditions where for example you used a streptomycin-resistant mutant for the feeding at the time when the animals were receiving streptomycin and their normal flora was being rapidly diminished. Almost anything that comes out of such experiment is bound to be, to my mind, extremely interesting.

I would fully echo the concern that you feel that the most interesting and novel situations in the new antibiotic program might be lost in the shuffle. I think the whole implication of what is meant by the cream having been already skimmed off this situation by the large companies means that you have to scrounge around with somewhat more difficulty in the skim milk. There is always bound to be at least some inverse correlation between uniqueness and quantitative facility. I know that you are not going to worry much about your situation until you learn whether or not Felix will be staying on with you. I think you will be interested to learn that Tom Nelson has taken a job in the Botany Department here working with Fulton Skoog on the so-called lakes project. As you know, the development of blue-green algae blooms on our local lakes has been a matter of very great economic concern so that there has been a very extensive program of the study of the physiology of these organisms. Tom is going to be involved in the development of techniques for the mass culture of the blue-green algae but one of the main preoccupations or end practical results of this Lake project is certainly going to be the development of means of inhibiting the growth of these organisms. It just occurred to me that if quite the right antibiotic agents could be produced which would be effective enough to make it worthwhile to use them on the tremendous scale required here, there would be a very large market for that kind of antibiotic, that is to say, algacidal agent. Of course, one shudders to think of the ecological upsets that would be the consequence of such interference in the normal cycle of lake growth. At any rate Tom is quite well fixed up here, at least for the academic year. There is no promise on either side as to whether he will continue and in the event that Felix does leave I think you should certainly look to see whether he is available and suitable for a position in your outfit. On a long term basis however, as long as the subject has been brought up, I would think that Galen Bradley would be a more effective member of your research group. However, I am not going to recommend him to you or vice versa unless your company policy would be such as to make it possible to assure Bradley that he could spend at least the larger part of his time on basic research and I realize that this may be much-too-much to ask at the present time. Still I don't know how you are going to be able to get the best possible personnel to handle even the genetic application of actinomycete genetics unless you can make your openings attractive on this basis as well.

There is not a great deal to tell you about the progress of our work here, clearly Simonti has gotten a good deal ahead of anybody else. I think his mere demonstration

of the existence of recombination in actinomycetes ought to be sending thoughts into the minds of a great many people. Galen had gotten about as far along technically I think as Simonti did. We have developed a fairly good collection of auxotrophic mutants in various strains of streptomycetes which were isolated from the soil with reference only to their convenience in laboratory use. The technique that was used for developing these auxotrophs was simply that of replica plating of the sporulating colonies after UV irradiation of spore suspensions. As far as we can tell in the species that we have considered, the conidia are strictly unique nucleate. Combinations of different auxotrophs under conditions of rather stringent selection do result in occasional prototrophs being formed. This is about as far as it had gone during my earlier essays into this particular area. Galen has gone over most of that rather more carefully and recently we have been able to prove unambiguously that these prototrophs are in fact heterokaryon because when single hyphae are isolated with the micromanipulator they give rise to cultures which are prototrophic themselves but which throw off exclusively parental auxotrophic spores. In the vegetative state, however, heterokaryotic mycelium, once it is formed, is relatively stable as such and this does leave the door open for the possibility at least of exploring useful applications of heterokaryons. So far we have not run into anything that we could be sure was recombination in a way that Simonti does in the path he has done so easily so this must be very largely a matter of strain difference and it would be impossible to predict whether such recombination phenomena would occur in which strains.

The only reservation that I continue to have in discussing these actinomycete problems is that you not tell me anything that you would not want me to pass on to Galen because I obviously cannot hold him to any confidence on these matters if other people ask about them but I think we've gone over that question sufficiently so that we know pretty well where we are. Of course I would be delighted to hear whatever Felix has to say about his own genetic program. I can certainly sympathize with the almost necessary slowness with which it proceeds. We've had our own experience with that. How, by the way, is Ethelyn Lively working out? I hope she is an asset to your organization.

Well I suppose the most important immediate question is the technique of induction and I hope you'll keep me informed as to whether you consider it worthwhile to try some of these modifications and how they do work out. I'm really convinced that you can do a lot better with the system.

By the way, my suggestion on the use of blood agar was not quite along the lines that you seemed to have implied it for. There would of course be no particular advantage in using blood agar as compared to nutrient agar for simple quantitative assay of plaque titers. It turns out however that the mere process of lysis, that is to say, of induction, appears truly as the hemolysis and I have thought that you might be able to get a useable measure of induction simply by adding some blood to the tubes in which the induction was taking place and seeing the extent of hemolysis that went on there. This would make it quite unnecessary you see to do further platings in order to count the number of phage particles that would be released. Whether this is still worth a trial or not, I don't know, but I think it would be amusing if you would simply do an experiment along the following lines. Grow some K-12 in complex medium, wash it, treat it with UV light, and I would say a dosage equivalent to let's say 60 seconds of a 15 watt sterile lamp at a distance of 50 cm and then put the washed cells back into Penassay medium under conditions of aeration together with a certain amount of blood. You, of course, will have a control using cells which have not been exposed to UV light and then after a couple of hours sediment out both the bacteria and the red cells and see how much hemoglobin has been released as the supernate. I've never done this experiment in just

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this form but my prediction is that, correlated with the induction of the bacteria, there will be a substantial release of hemoglobin which could be looked for colorimetrically. Of course it would be impossible to do a test of this sort with broths that were themselves hemolytic, but it would seem to me that an indirect test of this kind might let you do away with the rather complicated plating procedures which are needed for counting the amount of phage which is released by induction. I am by no means certain what this hemolytic effect actually is. I suspect it reflects the fact that the bacterial lysates are themselves at least for awhile hemolytic but this is a question we really should examine somewhat more directly. And we may conceivably do an experiment along these lines ourselves at some future date.

Goodbye for now.

Joshua

JL:jlg