Joshua Lederberg
Madison, Wisconsin
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Dr. Joseph Lein
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Dear Joe:

I am very sorry that my last message should have been mutilated. I suppose I shall just have to be more careful in my mailing procedure. I will do the best I can to recapture the main points that were included in that transmission.

You may be interested, by the way, that the record has a good deal more fidelity than is possible to capture by means of the very small speaker in these dictaphone machines and you can get very respectable results by playing them to any audio type of equipment. I don't know how much of a high-fi fan you are, Joe, but I was interested to hear how life-like a transmission can be gotten. This particular recording happens to be made through my own microphone and amplifier and I'll be interested to learn what your secretary says about the relative intelligibility of my recording.

First of all, you will by now have received a set of three Fla⁻ and two paralyzed strains of Salmonella. Excuse me, there will be three of each according to my notes. This is probably more than is worthwhile testing in any one bunch but you may find that some of the cultures are more or less suitable than others. I have not paid very close attention to several of these cultures recently and therefore you should subject them to single colony purification and testing before doing anything more with them. You will be able to see for yourself which of the cultures is sufficiently stable for optimal use under your conditions. Another consideration is that SW666 and SW1157 carry Fla⁻ factors which are closely linked to one another and these may therefore conceivably represent more closely related physiological defects than in relation to SW1153. We have no information on the genetic linkage relationship of the paralyzed mutants except that all six of the cultures are distinct from one another. Therefore it will be important to look out for transductional effects in your experiments as well as physiological restoration and function. This remark is especially pertinent if you are known to use mixed cultures in doing multiple screening. Otherwise I do not anticipate that you will have any difficulty although, frankly, I will be rather astonished if you can find the meaning of this destroying effect in these cases, that is to say, which represent a more than ordinary accomplishment and therefore one whose success can hardly be predicted. On the other hand, the possibility of finding agents which will inhibit Flagella formation or function is much more plausible and I will therefore be waiting to hear of some positive results of your preliminary trials on that system.

I have been waiting to hear from you with regard to a suitable lucite cell for some preliminary experiments on chemotaxis, but meanwhile I did a few more trials myself and have been rather puzzled to be unable to reproduce the phenomenon in a closed glass tube. I don't know exactly what's behind this and it might take some more looking at. However, the following design is something that you can quickly and simply do for yourself, although its main drawback is that it does not lend itself very well to quantitative procedure. I've been using the following technique:

On a clean, ordinary glass slide, add a cc or two of mineral oil from a dropper or glass bottle until the slide is more or less uniformly coated. Then with a

drawnout, fine, capillary tube place a small drop, about 3 or 4 mm in diameter at most, on the surface of the glass under the mineral oil. The culture should adhere to the glass and spread out under the oil enclosing it. This constitutes a primitive oil chamber. If you use a very actively motile Salmonella culture, you will then get an interesting effect if you touch the edge of a drop with a micro-pipette or a needle carrying a small amount of a solution of phenol of say 0.1% concentration. Within 5 minutes many of the motile cells will have migrated away from the point of contact of the phenol and have collected on the opposite side of the drop. This is the basic chemotaxis response that I've discussed with you and it would be, of course, very interesting to see if there are chemicals which modify the "steric ability" of the motile bacteria. It might be helpful to use a solution of phenol already made up with bacteria in order to avoid any possible confusion of migration with dilution of the drop with a clear added solution. The experiment I have just quoted you is just about all I am going to with this particular chemotaxis system and I wish I understood why it does not give nearly so startling a picture when the same experiment is done in capillary tubes.

During the last several weeks, Bob Wright spent some time playing around with the red, arginineless mutants which he finally got from Herschel Roman out in Seattle. From our experience, we would both say that this is not a very satisfactory method of screening for petit-producing compounds. The accumulation of red pigment apparently has a negative selective effect on the cultures so that they are continuously accumulating large numbers of various genetic kinds of light variants. These would be of very considerable trouble. It is possible to use this procedure for demonstrating effects of compounds like Acriflavin but on the whole I think you will have a much simpler procedure to use ordinary plating methods on the wild type cultures and relying on colony size as your final screening effect. This is not at all difficult to do and almost anyone of the large number of stock diploid yeast strains should be quite competent for this experiment. Of course you have the advantage of being able to pretest the system by the use of Acriflavin.

Another comment that I had emphasized in the record was a punctuation of my previous remark about the plausibility as desirability of looking for an anti-glucose. There has been a little work on screening for anti-lipolytic agents for use in the control of dental carries but none of it seems to amount to very much. On the other hand, there appears to have been no fundamental study on the mechanism of action of Phlorizin, a compound which was much used in the 1920s and 1930s because of its specific effect in preventing the renal reabsorption of glucose. The same compound has been noticed to have an inhibitory effect on the absorption of glucose from the gut and it might be that this would be a starting point for a rational approach in securing a desirable compound. The irrational approach is of course equally desirable, namely, the screening of your various antibiotic filtrates for activity of this kind. One of the first questions one would want to know is whether there is any microbiological system in which an anti-glucose effect of general utility could be detected and for this purpose it would seem to me worthwhile at least to do some preliminary experiments with Phlorizin on one or another microorganism. If you can give me some idea where to get hold of the stuff, I would even be interested to take a quick look at it myself, as I do not know of any literature on microbiological activity of this glucoside. Whether or not the compound works on Escherichia coli, it should also be tried on Protozoa and algae, for example Tetrahymena and Euglena, to determine whether it has a distinctive physiological effect there and I can very well envisage (A) that there will be glucoside occurring in Actinomycete filtrates if it has interesting activity along these lines and (B) that with suitable modifications Phlorizin-like compounds might be of considerable clinical importance.

In a previous letter I mentioned a rather obvious kind of design by which anti-glucose activity might be picked up with Escherichia coli. I would repeat again that the most likely agents for such activity would be found in synthetic modifications of the natural series. Again, such compounds are by no means exceptional as Actinomycete products.

I think I might also have mentioned to you that Dr. Bradley will be going to the University of Minnesota starting this fall in the department of Medical Microbiology. He expects to continue his studies on the genetics of Actinomycetes.

Pontecorvo was visiting Madison last week and as you probably know will be spending the summer at Cold Spring Harbor just in case you might be interested in trying to make contact with him yourself. Ponty gave us a very complete account of the genetic studies with Aspergillus and Penicillium and there is no reason in the world why one can't go ahead with a comprehensive genetic analysis and breeding program if only there were something worthwhile to be gotten from it with these particular molds. As you may already have noticed, there is a paper in the last issue of the J. General Microbiology by Sermonti which concerns the genetics of the effect on penicillin production. Although all of the mutants which Sermonti describes here appear to have been allelic, I doubt that he had really covered the situation completely.

As best as I can recall now, I do not have any additional suggestions at the instant for microbial test systems but I suspect you already have about as much as you would care to handle anyhow. It seems to me I did have some fancies with regard to systems worth testing on mammals but they seem to elude me for the moment and in any case are perhaps not too pertinent to our immediate task. The most dramatic of these perhaps has to do with the possibility of facilitating homo-grafting, a point which was excited, at least partly, by a recent note in NATURE concerning success in implanting part of the lymphoidal system of a rat into a mouse that had previously been treated with X-rays. This is part of a much larger question of acquired tolerance to transplantation and it would take quite a while to go into it here, especially as I don't know whether there is much point in bringing it up with you now. I would urge that you and your colleagues give this particular area very careful scrutiny, at least in my own opinion it is one of the more promising openings for drastic changes in medical technique. I had in mind the vague possibility that it might be possible to discover agents which would scratch the specific antibody reaction to transplant long enough that (a) these could become established and (b) that they could in turn induce some type of acquired tolerance on the part of the host. Some time when we're together I'd like to go into this in more detail with you, perhaps also with anyone else in your organization who would care to listen. As part of this story it seems to me that it would be quite worthwhile even right now looking for agents which can modify the action of complement on synthesized cells. Of course it would be a very simple test based on the prevention of hemolysis, or if you had to stick to a microbial system on the prevention of bacteriolysis by immune serum plus complement. Possibly along related lines you might find that antibody reactions against protozoa cells will more closely simulate the effect of antibodies against mammalian cells in transplant and these might therefore be of equivalent value. I think in previous discussions we had already discussed such physiological directions as are suggested by the blood brain barrier and the effect on its permeability which as far as I am concerned stems only from a popular account in the SCIENTIFIC AMERICAN and I hope that Amel has been giving very serious consideration to interesting the armed services in the anti-perspirant property of some of your drugs. You will of course have seen a recent area of interest in some synthetic replacements for

insulin, for example, crinase, and I assume it will have already occurred to many people in your organization to simulate this particular effort in one or other direction.

Meantime, I would be delighted to hear what you have been able to find out with regard to the modification of motility by various agents and what you think, for example, of the thiaminless unbalanced synthesis problem which we have discussed previously.

That does it for now, Joe.

So long,

Joshua Lederberg

JL:jlg

May 15, 1956

Dear Joe:

Postscript:

Just as a fantastic idea, what do you think of this: the host-specificity of viruses is almost certainly less than one commonly imagines; consider for example the plant & insect ambivalences, or Shope's story on swine influenza. Isn't it just possible that there are complexes in which protozoan (or algal or least likely bacterial) cells might serve as artifical hosts for "mammalian" viruses? Which cells? Which viruses? If you are going to maintain cultures of any other protozoe, just for the heck of it why not look to see if flu or mumps or poliovirus has any cytopathogenic effect.* It's the longest shot I can think of, but do you know whether this has been carefully investigated?

*or better, but more trouble, whether the virus is maintained or increases.

J

FPS: As an antidote to the foregoing, have I brought up with you the feasibility of a screening program to looking for bugs or enzymes that can split viruses into their protein and (relatively noninfective) nucleic acid components? Now that relatively pure preparations of TMV, flu virus, etc, are readily available it would be very plausible to enrich for organisms that can use these materials as sole carbon source, or N, source; it might also pay to have some ribonuclease present which is inactive on the intact nucleoprotein, in case this particular enzyme is lacking in the bug. Do you know of any parallel searches (since Dubos and the pneumococcal polysaccharides)?

J