MEMORANDUM

BRISTOL LABORATORIES

NOTED
MAY 5 1955
A. GOUREVITCH

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lederberg

DATE April 10, 1955

TO J. Lein

SUBJECT New Antibiotics Screening Program - Consultantship Arrangement

C.C.

Hello Joe:

This is dated April 10th and is in reply to yours of April 5. Your record was largely in the nature of comments on my own and leaves me not a great deal that needs reply. I am glad to have the information and some of the references that you did give me, and thank you for it. As this consultation goes on I get the feeling, and I should be surprised if it weren't so, that I am getting a closer and closer understanding of what you're about. Of course the time will come when distance will reach the point of diminishing returns and the very fact of being close to your problems may somewhat diminish my usefulness but of course I'll do my best. I hope you won't take my every suggestion too seriously. Your description of my function as a gadfly is, I think, more appropriate than that of a delphic auricle. I was a little surprised therefore that you paid very much attention to the notion I indicated of looking for high production of accumulated intermediates as a first step. I mentioned this only as, so to speak, a logical sequence, well understanding the probable technical barriers. However, I'm going to continue to mention things which to my mind appear to be technically impossible because it seems to me that my function is not primarily that of technical evaluation; that is really yours. If I took it on myself to decide beforehand that something was or was not likely to work I might be depriving you of ideas directly or indirectly which might lead to some useful application. In this particular case the fact that the low production of antibiotics seems to preclude that looking for them in the first instance by any method other than their biological activity - well that seems very reasonable but I would still keep in the back of my mind the notion of how worthwhile it would be to have some independent method of identifying such substances.

I had a chance over the week-end to look again at Waksman's more recent opus on Actinomycetes and Their Antibiotics and was of course greatly impressed by the large number of antibiotics that have already been described. It strikes me that your main problem must be not so much the discovery of anything new but the verification that it is, in fact, new. I was not very deeply aware of what you had in mind by way of your punch card system for the classification of your antibiotic broths but it would seem to me that you do indeed face a difficult problem in simply assessing the novelty of anything new that does come up. This is one of the things that I will want to talk to you about in greater detail when we can get together personally as it seems to me a rather too complicated affair to go over at this distance.

As to the general question of the natural function of antibiotics, in a way I might say that I am in agreement with your own position on the matter that it really is an open question. I have not had enough time in the game to have any feelings in the matter, as you recognize yourself the arguments that you presented are by no means conclusive although they do point in the direction that you had indicated. I have been particularly struck by the statement I've seen in several places that antibiotics tend to be adsorbed onto the soil. If the usual methods of testing antibiotic activity would require extraction of the antibiotics this might fail whereas the adsorbed

antibiotics still conceivably might function in situ under certain conditions. As to the points of the low initial activity and the specific requirements under laboratory conditions one could reply rather facilely, I don't know how correctly, that these notions apply primarily to laboratory circumstances and what goes on in the soil might be a very different matter. It might also be to the point that the actual amounts of antibiotic required for effective competition as opposed to complete antagonism may be relatively small since antibiosis is by no means the exclusive mechanism of such competition. Under certain circumstances one may even imagine that over production of an antibiotic could be deleterious under natural conditions to the organism which is producing it. In fact, one can argue that this is probably true from the very fact that it requires laboratory selective procedures to develop high production by Actinomycetes. This same point of possible self inhibition on the one hand and the subtility of ecological conditions in the soil on the other, I think, can also be used to counter your final point that the antibiotics of limited spectra would be less likely to be useful to the Actinomycetes. Be all that as it may, this is obviously a rather subtle question. It may not have a uniform answer, that is to say, some antibiotics may be of some use to the organism under natural conditions and other not. As to whether they are the blocked intermediates of normal metabolites however I am rather more skeptical. I have been for a long time a very firm follower of the general notions of comparative biochemistry to the effect that one is very unlikely to find important metabolites which will be unique for any single class of organisms. If, for example, streptomycin were an intermediate in the synthesis of a generally important metabolite I would imagine that it would have been picked up before now in some completely unique class of organisms and as far as I know, for example, I think penicillin has not been picked up as a product of Actinomycete metabolism and conversely for streptomycin and the molds.

And finally to finish up this topic for the moment again, have you seen the Symposium on Actinomycetes which was held at the International Congress of Microbiology at Rome in 1953? There are a number of papers in there which will be of mutual interest and I would emphasize at the present one by Villemin, Lechevalier and Wakeman which goes into somewhat more detail on the production of antibiotics in the soil and where they quote some of their own experiments, for example, that an ether extract of soil had properties similar to those of Actinomycin. However, as I think should be emphasized, there is nothing contradictory between the blocked intermediate hypothesis and the ecological function as antibiotics, since these concepts may be looking at the same material just for different points of view.

The main thing that does concern me on the viewpoints that antibiotics are blocked intermediates of generally important metabolites is then why are Actinomycetes apparently so uniquely important as potential sources of new antibiotics? They were seized upon in the first place, I imagine, because of their prevalence in soil and more likely perhaps because they happen to work. I wonder, however, if your main interest is the question of finding materials that are new and untried why you don't leave the antibiotics altogether and go to screening other kinds of soil organisms. This would be the most likely method of picking up materials that have hitherto been ignored on the one hand and on the other your hypothesis of the nature of antibiotics leaves nothing uniquely favorable for the Actinomycetes as potential sources of them. From the point of view of laboratory manipulation, organisms such as yeast or bacteria would be far more convenient; there would also be a rather more cogent possibility of detailed genetic analysis and control of the results. The question may be how deeply you are convinced of this hypothesis of the nature of the antibiotics and of course in an industrial operation it is not necessary to commit all of one's investment to a single theoretical line of approach. I do feel the more strongly the longer I think of it however that the

most likely technique for obtaining hitherto ignored materials would be to develop organisms completely out of the range of the Actinomycetes altogether. If they have been so thoroughly screened on a random basis it would suggest that it may perhaps be time to look for antibiotic activity from quite unique sources. Otherwise I would appreciate your views on just why Actinomycetes are such organisms of choice. Now I do appreciate, this is a point that has startled me in my own experience, that the unique thing about Actinomycetes may not be so much that they are capable of synthesizing antibiotics but that they have the habit of producing large amounts of materials that they put into the medium. On the other hand I am not certain that this is something that properly could not be shared by quite a number of other organisms. It might be of some interest to test Actinomycetes more directly for a trait of this kind in an objective way, for example, by obtaining an Actinomycete which is blocked in some particular step synthesis of some amino acid for example and seeing whether its level of production of the intermediate without any further handling of the organism is going to be strikingly different from a comparable mutant of Neurospora or bacteria or yeast.

Another logical conclusion on the notion of the ecological insignificance of antibiotic production is that one could infer from this that there ought to be no particular correlation between this trait and other characters whose persistence in natural populations presumably would be a matter of some ecological importance, that is to say, I sense without perhaps being able to state it in a completely logical, rigorous fashion certain contradictions between this notion and the idea of the necessity of looking through unique kinds of Actinomycetes in hopes that this is the best method of finding new kinds of antibiotics. The blocked intermediate notion would seem to me to lead to the conclusion that any isolate which is not of immediate direct fidship with any other has an equal likelihood of producing some new compound and I find in this paper by Waksman some justification from this point of view in the great variety of compounds which have been produced by what has been classified as a single type of Streptomyces griseus.

At one point in your commentary you asked me to explain more carefully why I thought too rigorous selection was bad. I think a little bit later on you expressed the point very well yourself, namely, that if in your elective isolation program you bored too strongly on a single carbon or nitrogen source you would, of course, before very long be exhausting the total number of types which could be isolated on that source and then be reduplicating your efforts. That's all I had in mind. The more rigorous your selection the sooner you are likely to exhaust the existing range of organisms that could be picked up on a given medium.

In some earlier paragraphs I did mention the possibility of autosensitivity as a factor which may limit the natural production of antibiotics. I think this is something that may have to be looked out for in screening for mutants which will produce antibiotics. I was struck, for example, by Kelner's comment that many of the antibiotic-producing mutants that he observed did in fact form rather limited colonies and grew rather poorly generally and it seems to me possible this may be due to direct inhibition by the antibiotic produced although of course it is equally possible that this is the primary effect, namely, the metabolic defect of which antibiotic production is a secondary consequence. This may suggest however other methods of going after mutants with antibiotic-producing traits, that is to say, to look for self-inhibitory mutants.

Finally, to answer a couple of Dr. Gourevitch's questions, I hope in the first place that he hasn't expected more of replica plating for Actinomycetes than it can furnish for any other organism. Of course, when the velvet is pressed down on a colony of any kind of organism it picks up not just a single point impression but a small area and depending on circumstances and the organism and conditions etc. there is bound to be a certain amount of spreading of the growth from the initial size. That means that even with bacteria it is necessary to limit the total population on the plate to something of the order of 100 or 200 colonies if the colonies are moderately small and to ride with less than that if they do not develop to a very large size. With the Actinomycetes, however, we have had no trouble for a long period of time with replica plating and sporulating colonies for the purpose of obtaining auxotrophic mutants and I can hardly think of any situation whose demands would be more rigorous than that. The main thing I can suggest is to make your replicas as soon as all your colonies on the plate have begun to sporulate and in that way they do not get too large, not to press too hard with the velvet since only a very light contact is all that is necessary and make sure the plates are moderately dry when the transfers are made. I will also take the occasion to discuss a little more closely the role of PAV as a metabolite and as an anti-metabolite. The former is, of course, very well recognized and the latter has been rather obscure; it is not at this moment clearly known what the anti-rickettsial function of PAV is based upon. I can't help but feel however that this is an utter coincidence that the two functions have nothing to do with one another. Perhaps the strongest possibility that I am aware of for the anti-rickettsial function of PAV is that it functions as an antagonist of another vitamin that Bernie Davis has picked up, namely, hydroxybenzoic acid. At least I know that he has made that suggestion and I'm not well enough aware of the current literature to know whether it has found a better or worse basis since he first made it.

Well, Joe, I'll be quite ready to get down to brass tacks or to make any kind of discussion that you would like to do. I realize that things may get to be a little disorganized and will probably get more so if we go on to personal discussion. I hope not to be belaboring any particular point too often since my function here is to serve you rather than to convince you of the correctness of any conclusion that I may have come to and I want to be sure that I do the foremost most effectively. What I will do, I think, is collect the various and odd notions that perhaps have not been dissolved over a period of several months at a time and perhaps every 4, 5 or 6 months I'll prepare a fresh resume in order that you will have these issues readily at your disposal to act upon as you see fit. I appreciate that there are necessarily differences in lines of action in your set-up as compared to what we would do in a laboratory like our own and I'm not well enough acquainted with how you can run things to be able to interpose my own judgement on what you ought to do. As you suggest, however, my function will be to remind you of the things that ought to be thought about. I hope you never thought that a consultantship would mean that you would have someone else do your thinking for you. I think you are going to find the contrary; you're just going to be faced with so many more problems than you had before because your consultants never go into making decisions for you and his advice is bound to be of a rather oblique nature and as you suggest perhaps his main function is to help keep you on the ball which means that much more work on your part. Well I hope I won't have to make too many more philosophical justifications as we go on. However, barring these rather infrequent resumes which I will make as my understanding of the situation develops, I'm going to leave it up to you to take the initiative as to which particular topics we ought to explore in many details as time goes on. Obviously we could very readily go all over the map and we have to have some sort of rhyme and direction to the story which I'm going to leave for you to do until you give me the cues as to just what things

you want me to go after.

So long for now. If I sound just a little bit tired I hope you'll keep in mind that this is the beginning of the spring gardening season.

Josh

JL:mjl