MEMORANDUM

BRISTOL LABORATORIES

NOTED
MAY 5 1955
A. GOUREVITCH

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lederberg

DATE March 28, 1955

TO J. Lein

SUBJECT New Antibiotics Screening Program - Consultantship Arrangement

C.C.

Hi Joe,

Here are some further, I hope rather better organized, comments on the past assignment. I am also sending an outline of these remarks but I think some more casual discussion may help fill in the outline. I am still feeling my way around your thinking on this problem and trying to orient my own so these will not in any sense be the last words, as if there was any choice that they ever could be.

First of all, it seems to me that there are three approaches to the whole question. The first is the sort of muddled trial and error set-up which has been pretty much the pattern up until now. A second might be the theoretical approach, the one which Waksman has espoused on the theory, but not very much in practice, which is based on the question "What is an antibiotic?". The third is something of a fusion between these two and I might call an operational approach which is based on the retrospect on the successes that have been made and is an attempt on operational grounds to try and find what further paths there have been. The operational approach is, I think, reasonably well exemplified by your own program on looking for new kinds of Actinomycetes by elective isolation. Now I must admit that I will be somewhat prejudiced in terms of the theoretical approach to it. This is perhaps not the one that yields the obvious, immediate dividends but is obviously the only basis on which one knows what one is actually doing. Unfortunately I do not think that there is enough information presently available to answer the question "What is an antibiotic?", that is, from the point of the organism that is making it. You seem to be pretty well convinced that they do not function as antibiotics under natural circumstances. I have seen only a limited discussion of this question and what I have seen does not appear to be very convincing one way or another. Can you refer me to published sources for this concept?

It hardly seems to me sufficient to say that analyses have been made on certain kinds of soil or at certain seasons and that detectable levels of antibiotic have not been found on those occasions. The potentiality of producing antibiotic substances in sufficient quantities may be shared by the laboratory on the one hand and rather special habitats on the other. In fact, I wonder if there isn't a significant question in the notion that if it does have a factual basis that antibiotic production is limited in the soil but I must say that I am very much in the dark on this whole question.

A more curious point would be, if we followed through on the notion that antibiotics function as such under some conditions, what kinds of organisms might they in fact antagonize. It seems to me that the most likely competitors to which such antibiotics might be directed are other species of the Actinomycetes themselves. These, after all, are the dominant soil flora; they are the ones which are most effective competitors for the common ecological niche. I would also quote the analogies with the bacteriocins of other organisms of which, for example, the colicins, are the most outstanding for

there appears to be a considerable degree of, let's say genus specificity, in the inhibitory action of these compounds one from the other. From this point of view antibacterial activity might simply mean that the by-product based on metabolic similarity of other bacteria to the Actinomycetes which were the immediate targets with respect to the adaptive value of antibiotic function. If this is the case, then the mere fact that a given Actinomycete species appears to be dominant in a given habitat may be an index that it has some kind of competitive mechanism and if we are ever to set ourselves on a sound basis it might be worthwhile doing some exploration on this point. It seems to me that it could be most readily tested by mixing soil samples, culturing them together, testing that type which eventually does predominate from the mixed culture against the others that had been originally inoculated. There may be some real point in doing this kind of experiment since it may show the way by means of which the most active antibiotic-producing organisms can be automatically selected for. Now there may be nothing at all in this but if there were it might lead to protective methods not only in fresh isolation of active species but also of the means of selecting for them in the mutation experiments.

I would also be curious to learn if efforts are ever made to examine antibiotic production enough from the individual pure cultures of Actinomycetes which are isolated from soil samples but from crude inocula of the entire sample without purification. All of this comment will make sense at all only in the event that antibiotic activity is meaningful in the normal life history of the Actinomycete.

As a subsidiary question, have you ever concerned yourself with what it is that determines the natural biological profile with respect to the Actinomycetes in any soil sample. I have the impression that the Actinomycetes on the one hand are a rather broadly confident group of organisms but on the other that one is just about like the next so that there would not be very much to choose from among the group of them with respect to specific physiological characteristics. Have you any impressions on this? The obvious factors that come to mind in suggesting the determination of the biological composition of a soil sample would be the chemical nutrition, the physical environment, temperature (its variability and moisture in the same) but there leaving general chance factors adrift for the whole question of antagonism on the one hand by other Actinomycetes and on the other by other microorganisms as well. I'm not proposing that we have a complete solution to all the problems but I do suggest that any program in such as your own hinges on these in such a way that one should always be thinking about them. This has particularly to do with your question of the isolation of new and hitherto perhaps overlooked forms by the methods that you have indicated. Regrettably the basic information that one would like to have is essentially lacking because of the orientation and the research on Actinomycetes that has been effective over the past several years. At any rate the one suggestion that does come out of this is that if antibiotics do potentially function as such in the soil, then the likely targets for their action are perhaps other Actinomycetes and that this might in some way be used as the basis for selection procedures for the improvement of antibiotic function.

Now the alternative proposal that antibiotics are miscellaneous metabolites is, of course, not really exclusive of the first notion because in any event that's what they are. One would however substitute metabolites which function as antibiotics in the soil for the notion that these are metabolites without any known function whatsoever and whose antibiotic activity made them be presumed to be more or less by way of an accident. I find this rather difficult to believe, frankly, but in the absence of any particular evidence one way or the other it's an approach that should hardly be neglected. As far as

I can see it makes very little difference which way you think about it for the purposes of designing the mutation program. In any event antibiotics are metabolites and insofar as they are metabolites their production is assumed to be under genetic control. We, fortunately, already have the precedent of Kelner's work which does show that even in what appears at first sight to be a qualitative sense, the ability to form antibiotics is subject to mutational variation.

If then, this program is essentially a search for miscellaneous and bizarre new chemical structures, conceivably it is a mistake to preselect them first on the basis of antibiotic activity. Instead there comes to mind what one might call the Raestrick approach, that is, one which recognizes the wide diversity of organic compounds which may be produced by Actinomycetes. One might then attempt to screen Actinomycetes not in terms of their producing an antibiotic in the first instance but in terms of their producing large quantities of new organic compounds. These, then, might be screened in some rather broad way for their potential antibiotic activity; this procedure would only be economical if there were some easy method of first determining whether large amounts of unique, new metabolites are being produced otherwise it would, of course, be more economical to look for antibiotic activity in the filtrates in the first instance, keeping in mind however that insofar as this is the standard operating procedure of the other companies it would be less likely to uncover quite new developments. I, unfortunately, cannot readily suggest methods of detecting, and more particularly characterizing, even moderate or large amounts of new unknowns. The only thing that comes to mind immediately would be some technique of chromatography of filtrates. With little experience, I suppose, most of the familiar compounds would be spotted and discarded. The question might be how to locate a compound of unknown composition. You'll need some more help from the chemists on a point of this sort but I might think on the one hand if chromatography on glass or some such compound with any general method of testing organic compounds, charring or perhaps (well this is possibly prohibitively expensive) the use of autoradiography to test the accumulation of particular spots. Off hand though, I would have to admit that this kind of reverse screening would be unlikely to be even nearly as efficient as the direct method of antibiotic detection but it conceivably may suggest an angle that hasn't occurred to me yet.

March 31, 1955
Continuation

Hi again Joe,

I think I had left off with the remark that one might have a more straightforward approach to the whole question of detection of new products if these were in the first instance detectable without regard to their biological activity so that this could be left for an entirely objective and independent evaluation. I only mean to leave you this as something to think about because, frankly, I have no immediate suggestions on how this could actually be accomplished in a screening program. Now my next remark might be considered new.

Regardless of whether the antibiotics function as such in the soil there is no question but that they have to be considered metabolites be they intermediaries in the synthesis of other compound or themselves happen to be the end product of a considerable sequence. I don't think it very much matters one way or another and I'm not sure one could really precisely define the difference between these two notions. At any rate if these are

metabolites it then, of course, follows that their production is under genetic control and we already have the actual experiments of Kelner which show this possibility. I am still considerably puzzled as to what Kelner's experiments actually mean, that is to say, what happens when you discover a mutant which now produces an antibiotic which had not previously been detected. I should say in the first place that I am extremely skeptical of being able to manage what I might call true genetic novelties. Now, of course, such mutations, we might call them neomorphic or mutations for new enzymatic specificities, very likely, in fact inevitably, must have occurred in evolution. However, the impression that I have gotten from all of the work in this direction that I have done has been that if these occurred at all they must be extremely rare and that most of the mutations which one now observes can be considered as falling into one of two categories: the first is the removal of the effective functioning of an enzyme or enzymes or second a reverse mutation, that is to say, one which puts the original system back in balance. Now on truely aprieri grounds that is exactly what one would expect. It would be much more difficult to synthesize or create an entirely new system than it would be on the one hand to induce some specific damage in the existing system or on the other, and this perhaps even more rarely, to repair damage that had been done previously. This is a very different kettle of fish from mutations whose effect would be to create entire novelties in the formation of enzymes. Now I don't think that Actinomycetes are the most likely organisms in which to look for evidence along this point, but it may turn out that they will be in fact useful in just that connection. I would point however to the fact that, for example, in sucrose negative strains of Escherichia coli I at least have found that essentially it is impossible to obtain mutants which would ferment this sugar. On the other hand, the lactose negative variant strain that had been obtained from wild type in their turn have, as a rule, a well-recognizable capacity of reverse mutation or of responding to suppressive mutations at other loci. This kind of experiment has been done many times and in essentially every case where an apparently new function has been noticed it would be at least as reasonable and in the majority of cases much more reasonable to argue that there had simply been the reparation of previous damage either by reverse mutation or by other changes in the background gene type which had a comparable effect. This argument is based on only two points however; one the apriori notion that the formulation of an entirely new process would be expected to be a much less probable event than either of the other two that I had mentioned and second the actual fact that in most cases naturally occurring deficiencies tend to be far more stable than those which are obtained by laboratory mutation, the exception to this rule being most readily explained in terms of previous mutation. For example, in the Salmonella group it is not at all infrequent to find tryptophane-dependent organisms which in their turn will readily revert, so to speak, to autoauxotrophs, types not requiring tryptophane. Since the general pattern of nutrition in the Salmonella group, however, is autoauxotrophy it is most convenient to regard these acquisitions of tryptophane synthesis as effective reverse mutations rather than the novel experience of first learning how to make tryptophane.

Having stated this suspicion that the development of the ability to produce antibiotics by mutation is not likely to be a completely novel pathway, let me indicate what I consider some of the more likely alternatives to be. The first is, of course, that we must distinguish between synthesis and excretion. Now we have no convenient method of determining the synthetic capacity of a given strain of Actinomycete except in terms of the material which is poured out into the medium and on the one hand we might have to consider the fact that different media and therefore different gene types are going to have a profound quantitative influence on the extent of synthesis and on the other that the very fact of accumulation and excretion might be a function which is quite different from that for the initial synthesis. The second possibility is already implicit in what I had

said before and one that is, of course, obvious that these are not new functions in any sense that are being brought out by mutation but the simple quantitative improvement on any grounds of a function which is already present. This again would be something that would be rather difficult to settle definitely without a considerable amount of experimental work although one can imagine use of tracer recovery experiments, isotope dilution, and what have you, in order to detect minimal amounts of previous synthesis.

Finally, while I would consider it a most improbable event indeed that a true evolutionary novelty in terms of the development of a new pathway, usually involving a number of enzymatic steps, could be controlled in the laboratory, it is, of course, conceivable that there will be mutations which will influence rather slight changes in specificity and might therefore lead to derived products which on one ground or another might be more desirable than the original. I have in mind here, for example, the diversity of related penicillins. There the balance between one kind of penicillin and another is certainly under genetic control and it is even conceivable that there might be mutational changes in the specificity of a single enzyme which would result in a qualitative sense in the ability to form some new and distinctive antibiotics. I must state, however, that as far as I know there are no good clear cases of changes in enzymatic specificity as the result of a laboratory mutation in any material. Most mutations that have been examined have concerned the conditions or the extent or the mere ability to which the organism can respond by making a given enzyme or not and changes in specificity of enzymes have been extremely rare, if indeed there are any clear-cut cases. The best example is perhaps Moss and Davis on the pantothenate synthetase in Escherichia coli where temperature-sensitive alleles have been picked up and have been shown in turn that temperature-sensitive enzymes are involved. It would be a very difficult thing to determine whether this meant that the gene change had directly resulted in the change in the kind of enzyme produced or whether the organism had originally possessed potentiality of making both the normal and the temperature-sensitive enzyme where the temperature-sensitive allele stock had only the one.

As a final way in which mutation might influence the ability to form an antibiotic, it would seem to me that far and away the most likely event would be that mutations could interpose metabolic blocks which would result in the accumulation of intermediates in the synthesis of other compounds, these intermediates not ordinarily being detected, and it is of course quite conceivable that some of these intermediates will have interesting antibiotic activity. On these grounds, however, one would be tempted to emphasize in one's search stocks which had already produced a variety of complicated end products in the hope that there will be a sufficient variety of well-used pathways that blocks at any one of a number of points might lead to the accumulation of any of a number of distinctive compounds. That was one of the points that I had in mind when I suggested using organisms which were already producing large amounts of some distinctive end product. You are, of course, taking the chance that whatever that end product may be that none of the intermediates leading to its formation will be of any particular interest. That was another reason for wanting to have other means of detecting those stocks which are producing large amounts of complicated molecules since whether or not the end products are antibiotic intermediates in their formation might or might not be.

On the whole, these theoretical preconceptions give me the impression at least that the entire operation may be very chancy. The basic reason why we suggest going on with it is the hope that in the very process of continuing along this approach that other

notions of the concepts will crop up which will in their turn be of more immediate use. If indeed the major effect of mutational blocks is going to be to interrupt the synthesis of compounds whose pathways are already well established in that strain, then the obvious conclusion is that it would not be worthwhile to concentrate too much effort on any one strain since the particular pathway which is most vulnerable or most effective in one strain may not in fact have any intermediate station which would be of any particular interest to you.

The kind of thing that one might most profitably expect from mutational programs is, unfortunately, not the most technically feasible. I would be willing to bet as an example that by mutational operations one could obtain from an aureomycin-producing organism derivatives which would produce either terramycin or tetracycline in view of the close chemical relationship of these compounds one to the other. What I would be most concerned about is how exactly one would readily detect such changes. Of course here one has the advantage with aureofaciens of the yellow color of the aureomycin which might lead to the detection of colorless variants which still retain antibiotic activity. I wonder, in fact, if this kind of experiment has not really been done. Perhaps this is something I shouldn't delve into too closely. There would not as a rule however be any convenient method by which one could pick up such minor deviations in the character of the end product and at least for basic reasons of chemotherapy there would be only a very few situations in which such deviant end products would, in fact, be strikingly useful, though of course there's always the chance of augmentation of activity, reduction of toxicity etc. These however are just the things which would be most inconvenient to screen for on any large scale.

This seems a convenient point at which to leave what I have called the theoretical approach and take up what I might now call the operational approach which is based essentially on a purely impirical consideration of the details of procedures which in the past have given useful antibiotics and what modifications might be expected to give some further diversity. Now for this purpose I will simply take at its face your statement that further random testing is not the procedure that your company wants to go into. I am not well enough acquainted with the details of the program to be able to comment on your statement that this would be an uneconomical procedure. The question is then basically how to vary the standard procedure in such a way as to have the highest probability of picking up new materials. I was particularly happy to note that you made no mention of the primitive cross-streaking method as the initial step in antibiotic screening.

My thinking on this matter is somewhat limited by my lack of appreciation of what the limiting factors are in your screening program. I have no notion, for example, of what proportion of your effort is devoted to the collection of samples or the proportion to the preliminary tests, what fraction of samples are sufficiently promising in producing any antibiotic to be submitted for further tests and so on and so forth. I think any operational analysis would attempt to review the components of the screening procedure on the one hand giving some notion of the amount of labor which is expended in them and the other the efficiency or the screening value to the proportion of the cultures which pass and so forth that do come through there. For that reason until I can get that kind of background many of my suggestions are bound to be somewhat specious.

Now most of your first assignment and discussion to me was an account of your interest in the use of special nutritional and similar selections as a means of restricting the range of organisms that you were considering. I am still, as I perhaps already

indicated at sufficient length, somewhat uncertain of exactly how much work you have to eliminate in order to accomplish something useful by this approach. If there were indeed a relatively small number of very prevalent types whose nutritional behavior was well known and which it would be desirable to exclude selectively in the first instance then I would say there would be no question whatever in the use of these specialized procedures.

If the previously known and unwanted types are however a considerable scattering of physiological forms of which some are likely to be included in this new, selective procedure and some are not, then I am not sure that you would be accomplishing very much in your specialized sample as compared to a random sample of the entire Actinomycete population.

In all I should say that your notion of special selection is extremely well worthwhile trying if only for the reason that it represents an approach that, as far as I know, has not been used before. However, I would be lending to some effort to validating the procedure that is in seeing what criteria can be set up to learn if it is worthwhile continuing with it or not and this may indeed be rather difficult.

One of the things that I am afraid of that you may run into is that you will be developing selective media for isolating a very few species and you will very promptly be running through the chance of whether they are or are not productive, and once a very small number of isolates obtained in this way had been tested the very specificity of your selection procedure may exclude not only the bulk of unwanted, well-known cultures but also new novelties which might otherwise have been of some interest to you. Instead you would be reisolating essentially the same few organisms again and again and again. Exactly how you would know when you had reached that stage at any given point I do not know.

For these reasons I think it would be well worthwhile to do a preliminary validation of these selective procedures by taking a fairly mixed group of cultures which represent the things that you don't want to have and seeing how they, in fact, would respond if they are put through the mill through your selective procedure. What I am a little suspicious may happen is that you will be picking up not only specialized types but new, reverse mutations, and what have you, of the existing populations. At any rate I would be particularly leery of any selective medium which was too rigorous in excluding too many different types. I don't know what the cost accounting factors are, as I questioned above, in your operations but I would suppose that if you are limiting more than 90-95% of distinctive species, shall I call them, that your selection may be too rigorous.

I think it would be more meaningful too if the agents that you were using for your selection were physiologically meaningful because I think this would have the largest chance of picking up a new kind of ecological variety or set of varieties of your Actinomycetes. The kinds of variables for use in the initial screening that have the most obvious bearing would be pH, temperature, selenity and Ridox potential, and I wonder how much work has been done based on the original screening of Actinomycete populations on media which are not optimal in these elementary terms for the majority of Actinomycetes. Here again, I think this could only be validated by means of a prior reconstruction experiment, so to speak, in which a group of a few dozen cultures which represent the things that you want to exclude are run through the mill to see in fact how they would bear up under the particular conditions. Of the things that I have mentioned, I think temperature (both high and low extremes) and particularly Ridox

potential might be things that have been to some extent overlooked by other investigations. To accomplish such the same thing it would seem to me that I would place more emphasis on thinking about where the marginal habitats of Actinomycetes are. It would seem to me that the optimal habitats are not the place now to look for new, distinctive, active species of Actinomycetes. In the first place these habitats have been well worked over before, in the second place they are more likely to be represented by fairly typical species. It would be at the limits of the habitats where the incidence of Actinomycetes may in fact be fairly small and where there may in fact begin to be difficulties in isolating any at all but I think one would have the most likelihood of picking up new forms simply because the bulk of work so far has been done with ordinary field and garden soils; one can imagine such things as manures, and muds, and lakebeds and river waters etc. I should also think that in such procedures it might well be worthwhile using preliminary mass enrichment culture techniques with or without competition from other bacteria as a prelimimary to the platings for the final isolation. I think this notion of using marginal habitats or using physiological conditions which fairly closely represent marginal habitats may be more likely to give you a wide variety of hitherto overlooked forms than with the use of too exotic carbon-nitrogen sources etc. simply because I don't know what the physiological-ecological meaning of the ability to use an exotic carbon source would be. If you could, perhaps, give me some notion of what you are using by way of exotic carbon and nitrogen sources I might be in a better position to evaluate what you are doing. More important, I would be concerned about what fraction of your cultures are coming through on such media. In some I have no bones at all to pick with you about this aspect of procedure. It does seem like a very worthwhile idea but one that would be somewhat dangerous to use as a basket for all one's eggs, so to speak.

Another approach to this question of getting specialized types occurs to me and one that may tie in with the theoretical as well as operational approaches and, that is, in many ways what one is looking for may be Actinomycetes which are at the present time sensitive to the major antibiotics in which you presently have no interest and which may be sensitive as well to a number of the other antibiotics which are not chemotherapeutically useful. Now such multiply sensitive organisms might thereby be expected to have been already screened for their production of the antibiotics to which they are sensitive. If then this screened group were then found to have antibiotic activity it would be a reasonable presumption that this was a new activity. Now, of course, it's much more difficult to screen for a rare organism which is sensitive to a given antibiotic than it is to screen for one which will be resistant to it. The only suggestions that I can make on this point would be to take advantage of such detection procedures as the marginal enrichment technique applied more or less in reverse in which limiting levels of the inhibiting antibiotics are used and one then goes after the ones which appear to be somewhat inhibited or perhaps more effectively the replica plating technique which would enable one to screen fairly large numbers of organisms against a rather large series of antibiotics. I have in mind here the success which Wakeman reported some time ago in the use of streptomycin to isolate streptomycin-producing organisms. Conversely, organisms which are sensitive to streptomycin would hardly be expected to produce it although I would keep it in mind. Stedola and others comment on an organism that they picked up which produces hydroxystreptomycin and nevertheless appears to be sensitive to it but I think this would be something of anomaly. This approach would only be reasonable if the preliminary screening and isolation tests are a negligible fraction of the total work involved and if the consideration of whether the organism is producing an already known antibiotic represents the most troublesome bottleneck in the procedure.

In view of the breadth of the discussion that you presented me with from this first assignment I think it might still be in place for me to make some general comments about evaluation procedures. My impression of the whole story of antibiotic screening, once given that a large fraction of Actinomycetes do produce antibiotic substances, is that one should be extremely circumspect about the criteria used for the preliminary evaluation of a given culture. Now I realize that in the early days of this game that people got very rapidly and much too excited about the very fact of antibiotic activity and didn't pay enough attention to the actual usefulness of their antibiotics as chemotherapeutic agents. However, I think one should be equally cautious not to swing too far in the other direction and overlook negative goals that have been passed by because they appeared superficially not to fit the standard criteria. I think I'll never forget hearing Michael Heidelberg in a lecture on chemotherapy tell us that he had in fact tried sulfomillamide back in 1918 as a chemotherapeutic agent in a routine survey an' although some antibacterial activity had been noted at that time it was rejected because it was not the sort of single-shot panacea in small doses that Ehrlich had prescribed as the goal of chemotherapy. Much the same thing happened again with mepharcan in the treatment of syphilis where there was the lag of some twenty or thirty years between the first observation that it had therapeutic activity but was rejected all that long time for what now appear to be extraneous reasons, these reasons being principally the size of the dose in absolute terms rather than in relation to toxicity that was needed for a curative effect. I think one has to keep in mind too the fact of history that the sulfonamides are also rather considerably antagonized by serum and that again it is a quantitative question that they have been very effective as chemotherapeutic agents. I would also keep in mind that penicillin has a similar binding capacity, that thiscapacity does however vary considerably from one minor variety of penicillin to another. This perhaps suggests a point for the screening programs, namely, to look for deviant end products or deviant antibiotics in terms of just this trait, that is to say, if you had just picked up an antibiotic that was very desirable in terms of toxicity and its <u>in vitro</u> antagonisms it would not be too much to hope that you might find modifications of it which would be less influenced by the presence of serum in the medium.

Well in this evaluation problem, I would first question what kind of antibiotic you are looking for. Now if you are going to put all of your guns on the objective of finding more useful broad spectrum antibiotics, then the rest of my comments will be of little use. I can't help feeling however that we are going to be more and more discouraged about finding many more of them. They are anomaly in the first place. I can't understand how they can exist because I am just amazed that any substance which can be so non-toxic for higher forms as is aureomycin will in fact find something in common among the great diversity of micro-organisms that it does in fact attack. If we only had some of the theoretical information as to how this discrimination can be made, we would, of course, be on a much sounder basis for looking for more like them.

My own guess, and of course it is not a very well informed one, would be that there will perhaps be a few more lucky breaks of this kind but that we are really going to have to rely more and more on rather more specialized therapy for individual diseases. From that point of view the greatest weakness of the screening programs as indicated here is its reliance on a single test organism for the preliminary determination and since this is something which probably most companies are doing, perhaps it would be the smart thing to do to emphasize multiplying the tests on a wider variety of organisms. Here again, I think of something where replica plating may be of some use because it would seem to me it would not be too difficult to use the antibiotic broth as the base for a

few plates of varying kinds of agar media and then one can use replica plating for the inoculation of a large variety of different microbial types against a single tested broth. At any rate I would be inclined to think that here more than any other way one would be likely to be getting on to territory that had previously been overlooked. From this point of view one can't help being reminded of a similar trend in the development of the synthetic antibacterials and I have in mind particularly the fairly specific agents against various protozoan diseases on the one hand and of course the very startling success of the isoniazid series against tuberculosis where it's my understanding that these compounds have very little effect on most other bacteria. If then, we could ignore the obvious technical limitations it would seem to me that the proper order of a screening program of antibiotics would be first to determine which organisms did in fact excrete reasonable quantities of some new compound. Second, to determine whether that compound was toxic which would probably rule out a large majority of the new isolates and then finally to determine what antibacterial activity, if any, this compound might have. And only as a last step we have the question of protection in vivo. Now in practice it may not be reasonable to conduct the toxicity tests in the first instance but I lay out a program of this kind just in the hope of provoking you to be thinking about ways in which in fact it could be accomplished. I have in mind the ultimate development of toxicity screening tests which might be greatly simplified over the usual procedure of injection into live animals. In practice as well, you may find it inconvenient to look for metabolite accumulation prior to the detection of antibacterial activity, that is to say, the one characteristic of accumulation may be more difficult to practice the test than the one among many characterizations of significant biological activity. With present procedures the most effective modification of the screening program would then seem to me to be the inclusion of a much wider variety of potential target organisms at the earliest possible stages.

The next point in the evaluation procedure that troubles me to some extent was the one based on inactivation by serum. I have already said enough in this direction to indicate the nature of my reaction to it, that is, it seems to me that it would be a mistake to automatically exclude an otherwise promising compound simply on the basis of apparent reversal of activity by serum. Perhaps you have much more impirically founded data than I do in order to rebut that question. It would seem to me that serum inactivation might be partially a question of absolute dose which is not a terribly important point unless one considers it from the economical point of view of cost of production of large amounts. On the other hand other measures might well be found which could mitigate that single effect. Precisely the same point of view applies to the further test in the evaluation procedure. It seems to me that you may be looking only for a very narrow range of materials if you look for antibiotics which will have dramatic curative effects against overwhelming infections. It would seem to me that would be something of more interest to the Advertising Department than to the Research and Development Departments at this stage in the game.

Finally, there is one aspect of antibiotic activity that I think should not be overlooked and that is the possibility that useful substances may be found which are not properly speaking chemotherapeutically active in their own right but which might function very effectively as synergists of other antibiotics. I have in mind antibiotic analogs of such things as PAS in the therapy of tuberculosis. This is going to become a very weighty problem with every antibiotic. The whole question of development of drug resistance is tied up with this. It would seem to me that there may be very real room for substances which have by themselves very limited activity but which may potentiate the activity of other primary antibiotics or which may help by mopping up the resistant survivors of the first one. I think it would not be too difficult to design at least a

few arbitrary in vitro procedures by which such activity, in combination with other drugs, could be tested although I realize that to do this on a comprehensive scale would be an overwhelming job.

I would include at this point some discussion of some of the kinds of basic information that are very sorely lacking and in the absence of which a lot of what is being done is more or less by guess rather than on any really rational scheme. In the first place, I would like to know much more about the function of antibiotic activity in santu in the soil and the role that this plays along with others in the selective determination of prevalence of Actinomycete types. Second, I would be curious to know what happens to antibiotics in terms of their metabolism by other Actinomycetes or by other bacteria. Again primarily in reference to the ecological factor mentioned above.

Finally, as a point of practical information in the mutation screening program I would be curious to know what evidence there is aside from Kelner's very dubious and limited work on just this point as to how diversely arranged antibiotics can be produced by mutation from a single culture.

At a more purely technical level of discussion, although the details I have to go on are somewhat limited, I have heard nothing that I could criticize. By proposing a mutation program I doubt very much that I could add to Felix's appreciation of how to induce mutations. Kelner's modification of the sandwich plate method seems entirely reasonable although one can imagine related methods based on diffusion through membrane filters and what not. I don't see any apparent advantage to them from this distance. One would like more basic information on which to base one's conclusions of what to use for starting cultures in the mutation program. In any event I think it would be a mistake to gamble on any single strain or in fact at this stage of the game to use any single criterian for the selection of strains, that is to say, I would have one group of strains in which in the mutation program was selected because they had no current antibiotic activity and would therefore be the most convenient for detecting new activities. I would use another group that was already accumulating large amounts of a well characterized antibiotic which could be excluded from consideration by the appropriate use of resistant bacterial indicators. And finally I would also use cultures which were producing large amounts of other end-products, pigments, or what have you, in hopes of picking up a wider range of blocked intermediates. I would also give some consideration to the possibility of the development of elective or competitive procedures for the enrichment of antibiotically active mutants based on competition either with the original wild type strain or with competition from other bacterial or Actinomycete species.

Now, Joe, all of this is, of course, a rather rambling affair. This is my first introduction to the whole story. I'm still getting some of my thoughts organized in it. I'm not always exactly clear what it is that you want to get from me. I'm going to leave it to you to set the agenda and to give me some restrictions as to the scope and the kind of discussion that you want to have. I am very anxious not to waste either your time or mine. At this stage of the game I thought I would put down all I had. If you give me instructions which will define the scope of this consultation I would be very happy to follow them. Perhaps all I will be able to do for you is to argue some concrete questions as they come up with you. Frankly, I think it somewhat unfortunate that the one laboratory which has set up a genetic consultation as far as I know is the one that probably has the least requirements for it in terms of qualifications of its present personnel. So long for now, Joe.

Josh