MEMORANDUM

BRISTOL LABORATORIES

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lederberg

DATE September 22, 1955

TO J. Lein

SUBJECT New Antibiotics Screening Program - Consultantship Arrangement

C.C.

Dear Joe:

I am sorry we did not get to see you at Lansing. We had originally tentatively planned to go as is indicated by the titles we submitted but we found that we had relatively little time after our Colorado trip and furthermore that we would have to drive around Chicago on Labor Day in order to make the meeting. The airplane schedules were limited on account of the holiday and even the more crowded and we weren't able to get satisfactory reservations. I am hoping that there will be an opportunity for a trip and visit during the time that you indicated. However, I cannot make any definite plans just yet because of the uncertainty about when the remodeling of our laboratory will begin and any traveling that I do hinges upon that. I will let you know what possibilities, if any, should materialize.

I was interested in your comments about the cancer meeting and my own general impressions are consistent with yours although perhaps not so extreme. I think there are a number of workers who are making useful applications of genetic concepts in the field, for example, Ted Hoshtra. It's hardly their fault if the transplanted tumor cells don't always display completely familiar behavior and it is indeed rather remarkable to see how plastic the chromosome content of tumor cells can be and remain consistent with the viability of these cells. This is something that was quite a shock to me when I listened to those meetings on the Ascites tumors in New York. If you are going into the matter of tumor agent screening in any large way, I would certainly recommend that you get in touch with Ted Hoshtra who is now not very far away from you in Buffalo at the Roswell Park Memorial Institute. I believe you asked me once to suggest the names of any other possible consultants who might be able to give you background material that was outside the realm of my own competence and I use that as justification for bringing up his name.

I still cannot agree with you that the somatic mutation theory of cancer is completely out. It seems to me still entirely possible that the first event, a trigger to the chain of events leading to the development of the malignant tumor, is an ordinary somatic mutation. Once the tissue cell has achieved a certain anatomy and rapid growth rate one might naturally expect, as one would in any population of microorganisms, subsequent changes to occur sporadically and in the rapidly growing cells of the tumor which were selected for antibacterial activity etc. that is characteristics of metastatic tumor cells, that is to say, the initial event could still very well be a somatic mutation of a kind which in a way releases the cell from its internal and external controls. It is quite apparent that there are many deep-seated genetic changes taking place later on of which the amuploid variations in chromosome number are the most obvious and the most dramatic. This may have importance only insofar as one might be somewhat less discouraged about preventing the initial step of tumor formation if it were not a somatic mutation.

You commented on the use of transplanted tumors in screening tests for chemotherapeutic agents in cancer. Whatever comment I would add would make very little difference since it

To: J. Lein September 22, 1955 New Antibiotics Screening Program - Consultantship Arrangement

is not easy to see at the present time what alternative systems could be used for screening purposes. It is quite obvious that a tumor which has been transplanted for any number of generations has been subjected to the most rigorous selection for its ability to survive the shock of transplantation, new environment and what not and in turn might be expected to be somewhat more resistant to interference from chemotherapeutic agents administered to the animal. On this basis it might be advisable to use for tests of chemotherapeutic activity tumor lines of not very great antiquity. George Klein has commented that it is now possible to preserve Ascites tumor suspensions by freezing and this might be a useful lead in the development of the standardized inocula for tests of therapeutic agents. I really don't see how you can conceivably get away from the use of transplanted tumors and the question will then come about what is the most sensible type of tumor to use in the same therapeutic agents. I'm sure that Ted Hoshtra would be much more knowledgable on such matters since he's working on that kind of material and I only read about it.

I was impressed by your report on the adoption of the notion of alkalating agents as a common factor in chemotherapeutic and radiomynetic activity. This is a point that has become increasingly obvious with the work of the British group at the Chester Hadey Cancer Lab in London but I was interested to hear that it had been so generally adopted. Of course I was using the work alkalating in a more general sense. I don't know what other terms to use that would include both substitutions of alkyl and aerial and acetyl groups which for present purposes are equivalent.

I agree with you that it would be quite useful to continue to look for radiomynetic agents particularly those of natural origin. The very fact that they can be produced by some kind of living cells would be at least preliminary evidence that they have some sort of specificity in their action which probably would be less likely to be true in reagents like acetylchloride which in principal have the same kind of base effect. It seems to me in fact rather remarkable that an agent with such potency would be a natural product and one wonders what mechanism the producing organism had to protect itself against such a compound as azaserine. Do you have any ideas about that since this question may eventually become of great importance aprime to developing the bases for differential toxicity by such agents.

It possibly will remain true that none of these reagents will be able to do anything that X-rays cannot. The one hope that you might have is to develop some other handle on these reagents that will give them a certain amount of differential activity. With regard to their relationship between the effects of these compounds on X-radiations, it seems to me that an entirely plausible case has already been built up for the roll of peroxyl or similar types of derivatives in the mediation of X-ray activity and I am a little bit surprised that there have not been more reports on the synthesis and testing of various kinds of organic peroxides for this type of behavior. Now some of these peroxides are of course highly unstable and almost explosive but others are relatively stable in aqueous solution. I would think this is something your chemistry group might be interested to think about unless of course I've simply overlooked a large body of work that may have been done in this direction. It seems to me there is every reason to expect that this type of compound will be as useful in radiomynetic action as would any of the other reagents that have been proposed.

I am sorry that you had such a disappointing result on the comparative resistance of strains B and B/r. I wonder did you check on these strains with ultraviolet light to

see if they fit the original description that Witkin gave of them? I have the impression but I can't document it that some workers have found that these strains in their hands are no longer as divergent in the irradiation survival as they originally had been. Whether or not it will be feasible to reisolate suitable irradiation-resistant variants is an open question. I agree with you that the question of correlated resistance in these radiation-resistant strains has been a somewhat muddled one and there has never been a very satisfactory explanation. I agree equally that it is very unlikely that these are coincidental effects but after all they conceivably could be considering that the mutants are always selective in the presence of mutagens. To my mind it is much more likely that the strains are simply cumulative, spontaneous mutations in this rather long time during which they had been maintained separately from one another. In any event the levels of differential resistance which were described are, as I recall, rather slight and inconstant from one strain to another and I think can probably be ignored altogether. The more important question is precisely what technique to use in differentiating the two. I am not surprised that inhibition zones would give a very poor differentiation for the reason that the diffusion and inhibition zone technique sets up a very sharp concentration gradiant so that there might be expected to be very little difference in the diameter of the zones even for rather different effective thresholds of activity. In such a case it might be more advantageous to compare strains by means of a gradiant plate technique which gives you a much more gradually spread out gradiant concentration of the agent. After all the use of inhibition zones is based on the very fact that at the boundaries of the zone there is a very rapid change in the concentration of the reagent that is diffusing from the center. Since the two strains would be compared side by side on a single gradiant plate it would seem to me that this could be quite a workable procedure although it would not lend itself to quite the mass scale of testing that a diffusion zone technique would. In the long run I would suspect that a differential inhibition test of this kind would be much more feasible for you than one based for example on the induction of bacteriophage and I will be very surprised indeed if there turn out to be any serious discrepancies in the range of compounds that would have the radiomynetic effects in these two systems.

You also brought up the possibility of using the induction of petit colony variants as a screening procedure. Fundamentally I think this would be a wholesome and productive idea but I am very dubious about the possibility of setting up a screening procedure that would be useable on such a large scale basis. One cannot really rely upon colony size as a criterion of this petit colony change despite the name that is given to it and you will note that in all of the publications on this question, Prussey always says that a sample of colonies was tested by using the knotty reagent or some such procedure. One of my students has been actively interested in this system so we can speak with a certain amount of experience. I suspect if you were screening for the induction of petit colonies it would be necessary to expose your population of wild type yeast in liquid medium to the action of the agent and then to plate this population out which would mean using a variety of dilutions on account of the variable amount of killing that might be expected and then combining both a colony size inspection with a sampling for tests of oxidative activity either with knotty reagent or with a simplified phenol red acetate medium that Lindengren described a few months ago in the Journal of Bacteriology, a technique which, by the way, we have found to be very useful. This sounds rather too laborious for a routine screening in the first event but it is something that might very well be worthwhile to look into on a selected group of antibiotics once you had them.

Now I have to tell you that a very wide range of reagents has this petit-inducing activity. In the first place they do occur spontaneously but in most strains at a relatively low

To: J. Lein September 22, 1955 New Antibiotics Screening Program - Consultantship Arrangement

rate. Acriflavin was among the first reagents to be tested and almost all the work that Prussey has done has been with that. However, we have found, akin with a number of other investigators, that ultraviolet light is also a very active reagent for producing petits. Finally there have been scattered reports that such things as X-rays, higher temperature, triphenyltetrazolium chloride and I think, but I'm not certain, nitrogen mustard, were also highly efficient in producing petit variants. One possible advantage of the petit system is that there is already some evidence that the scope of reagents which will have that activity is even wider than the usual radiomymetic reagents but for the moment I would be quite discouraged about carrying out that kind of a screening on any large scale for preliminary screening purposes. It would be worth giving the matter some thought since this would be a new dimension of biological activity of chemical agents.

Of the screening proposals that might be apropos to your aims of finding radiomymetic agents, I think the ones that already have been mentioned are the outstanding candidates. They would be: (1) differential between B and B/r or similar radiation-resistant mutants, (2) differential survival of haploid and diploid yeast and (3) induction of lysogenic bacteria. A fourth possibility would be the induction of easily scored mytotic or somatic segregens of heterozygous yeast. Mr. Right, my student who is working with yeast, will be making some yeast diploids which are heterozygous for a recessive red pigment character and these might be particularly useful for this latter purpose. If it does appear that these diploids are white and that they give rise to appreciable numbers of red segregens with moderate doses of UV light then we will send them on to you. I will also have ready some haploid and diploid yeasts if you would care to test them to see if they have appreciable differentials in their response to UV light. They are supposed to according to the literature but I don't know if it will be enough to suit your purposes. Again I think a gradiant plate technique will be almost essential in assaying any chemical reagent. If the diploid is not sufficiently different from the haploid it might be very well worthwhile to try to obtain some tetraploid strains of yeast which, for example, Roman, at the U. of Washington in Seattle, has been working on and which are reputedly much more radiation-resistant than the diploid. In any event I think the preliminary trials of these screening systems should be done both on the basis of differential survival and on the basis of some sort of gradiant plate assay. Unless the latter can be effectively worked up however, I can foresee that you will have some difficulty in large-scale applications.

As for the use of the W1485, i.e., lambda-sensitive SR indicator system, I will just have to wait to hear from you how that works out. I would by the way appreciate your sending me back the 1485SR so that we can keep it in our own stock for similar purposes. We had simply never bothered to prepare that particular strain since we did have some others which however had a number of other irrelevant markers.

Here by the way is a tip for picking up lytic effects. We have noticed that bacterial lysis is accompanied by very considerable hemolysis on blood agar. It seems to me just conceivable that you could mix a fairly small number of washed lysogenic streptomycin-sensitive bacteria together with 1485SR in excess and plate these on a blood agar base. On such a base you might conceivably get a very dramatic effect of UV light if this will induce even a fairly small proportion of the lysogenic bacteria. In effect this produces plaques on the lambda-sensitive bacteria in the background but these plaques can be very greatly accentuated by the use of blood agar and as a matter of fact I might

tell you that W1485 itself had been isolated originally by a plating of strain K12 on blood agar which had been exposed to UV light. It was noticed that there were some colonies that were giving hemolysis and when these were picked and replated it was found that the hemolysis was due to the reinfection of some sensitive colonies which had been induced by the UV light. The blood agar we used was, I believe, the ordinary 10% or so of either bovine or equine blood; I assume that human red cells would do just as well.

Since I assume that your aim is to avoid having to use a fairly elaborate dilution technique in order to estimate the number of free virus particles that are produced it might even be possible to adapt this hemolytic reaction for the assay of phage perhaps something along the following lines although we have never actually tried it. The lysogenic bacteria might be grown in the presence of or subsequently treated with an unknown reagent at a certain concentration in liquid medium. After a time is allowed for the release of phage in that system, the bacteria perhaps might be spun down. The supernate might then simply be mixed with a standard mixture of streptomycin-resistant, lambda-sensitive bacteria, that is the 1485SR, plus a suspension of blood in nutrient broth plus streptomycin and incubated for a standard period of time. The amount of phage liberated in the first test might then be roughly measured by the amount of hemolysis in the blood tubes. It may turn out in the long run that this would be even more elaborate than some kind or other of plating method. On the other hand this may suggest to you other ways of crude preliminary tests for massive amounts of induction. Next to a B vs B/r resistance differential, I would suspect the induction technique would be your best bet. If anything more practical occurs to me about using yeast in these systems, I will let you know and we will in any event send you some haploid and diploid strains.

The effect of Acriflavin is probably a pretty general one in yeast, the main trouble is to find strains which don't already have a rather high rate of formation in petits or strains in which the stock cultures are not already petits themselves. This is by no means rare. I do agree that there is at least a hypothetical analogy between the removal of the respiratory plasmids in the formation of petit yeasts and chemotherapy of viruses. This analogy is approximately as close as that of the disinfection of chloroplasts in such organisms as Euglena and here again is something if you were really going to go far afield, which I suspect you are not, you might want to think about. Were you acquainted with this, Joe, that is, that such agents as streptomycin are capable of bleaching Euglena so that you get the alga without its chloroplasts? This was described by Provosole, Hutner and Pitner in the 1951 Cold Spring Harbor Symposium. It has not been the subject of much work since. As far as I know, streptomycin is the only chemical reagent that has this effect although with some strains heating to high temperatures is capable of differentially inactivating the chloroplast. I do not know if there have been any experiments on mortypically radiomynetic agents. If you could get yourself set up for the culture of Euglena this probably would not be too difficult a test to run since it would just be primarily a matter of microscopic examination of Euglena cells to see if they had become bleached under the conditions of treatment. Just on the remote possibility you haven't run into this kind of story I have a discussion of disinfection experiments of this general kind in a 1952 review in Physiological Reviews of which I think I have sent you a reprint but I am not sure.

Of all the things we have talked about, I am perhaps most pleased at your interest in this question of substituting for the enteric flora because I think there is a tremendous opening for research, maybe too tremendous, for a limited attack but I don't know that

To: J. Lein | September 22, 1955 | New Antibiotics Screening Program - Consultantship Arrangement

anybody else is really going into it. This is the kind of thing for which germ-free animals would be very suitable experimental materials but unfortunately the developers of that technique seem to have sat on their hind legs and not done any other experiments with it.

I hope I did not give you too many mis-impressions about this occurrence of Serratia in the little girl. This infant had not been under any antibiotic therapy at all and the reason that I pointed out this case was that this was a strain that apparently was able to implant itself in competition with other enteric organisms even to the exclusion of E. coli and without any apparent harmful effect on the child at all. I would think that such a strain having been isolated under conditions that would seem to minimize the possibility of its pathogenicity and carrying the distinctive color marker, the culture might indeed be very useful as a test organism in replacement types of experiments. It probably would be necessary to at least try to make various kinds of antibiotic-resistant mutants from this strain in order to use them in your sort of experiment. There would be the advantage of the red color on the platings. I am not sure in fact that the pigment would be of any serious consequence even in a practical application should any justification for this appear since it will be apparent only in infants on their diapers and probably will not give very much trouble with ordinary evacuations. It is obvious that a tremendous amount of preliminary research would have to be done but I would suggest that this organism is as good as any. We will send you this strain along with the other materials that I mentioned. Several months later by the way this little girl was still excreting this particular organism only as a very small proportion of her total fecal flora.

Joe, I'm sorry that the very last part of the first record that you just sent was completely garbled. I think someone else must have come into the office at the time, at any rate nothing much came through the microphone. You had just started to say that you had read the Simonti article but I missed completely everything you told me about Felix's interest in the Actinomycetes and in the Penicillium so I may have to get that back again from you.

Yours sincerely,

Joshua

JL:jlg