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Dear Joe:

Congratulations to Pat and yourself. Do you really mean to take a vacation, or just one from the lab? Anyhow, I hope this message won't distract you.

You hardly need to explain to me any delay in our correspondence. The wonder is that you can ever break away from the routine— perhaps part of my job is to remind you to do it. At any rate, as long as I am on a retainer, you may rather have to explain to your bosses than to myself.

Thanks for forwarding the divers materials. It was something of a shock to read the transcripts— it always is; how could I ramble so? I hope and trust they are not that bad to listen to. I hope you will feel free, as I do, to rely on any method of communication. The main point is that I don't have a secretary I can call on for this work. But I will try to orgahize my material somewhat better. It is very kind of you to look out after the antibiotics. I did not mean for you to go to any particular trouble. Grisein sounds as if it might be worthwhile. We have had quite a bit of experience with strpptothricin already, limited mainly by uncertainty of supply. The cross-resistance with streptomycin, and the fact that a multistep system also seems to operate in K-12 has discouraged us from doing much with it as a marker, but sometime we may need to do more with it.

I am not sure how well I can help you with your request for purine-... mutants. I am sending what we have along this line, but with no warranty about the cultures which we have not examined for a long time. Demerec, at Cold Spring Harbor, or Bob Guthrie (at Roswell Park now) might be able to help you if these don't pan out, We have never looked into tetracycline resistance in any way. I amused myself by trying to guess what you're after:: don't bother to confirm or deny, but my hunch is you're going to look into accumulation and excretion of purine bases in relation to tetracycline synthesis.

As to the screening program, I wonder if you have concerned yourself why colonies should fail to grow on transfer; perhaps there is a group of organisms that needs special soil extract factors ? or other nutrients from exosymbiotic organisms? I was somewhat relieved at your experience with Nobel agar:: my own had not been as good as you had claimed; perhaps there is a good deal of lot-to-lot variation.

Thanks especially for the flowsheet and the programming data. I have not fully digested the same, and will defer comment.

One point does occur to me: 1) the large dropout between preliminary characterization of positives, and those kept for after time study. I was delighted that you do not discard the low-potent isolates at this point. It seems to me your competitors are most likely to ignore these altogether and that they might therefore represent the most unique material. But what to do about it? The weakest point in the whole program is how to handle these low producers. 1. You've already mentioned medium study. 2. Are you convinced that the extraction and assay procedures are beyond improvement, for handling these items? [On my next visit I will try to learn a little more about these from you]. 3. Is not this perhaps the most likely point to apply mutation procedures?

To enlarge on 3), I don't know if it would be hopeless to do a full analysis on every weakly potent culture. However, these are most likely o include antibiotics that have been overlooked by other workers. Two approaches are needed: a) means of screening out likely repeats even in low-activity broths, b) but regardless, a fair sample of these should be screened in at least a rough way— say 10 plates per cultures, from UV'd spores— for possible improvement in yield. It is uncertain whether there will be any marked correlation between increasing the zone of inhibition with Kelner's method and improvment of yield in liquid fermentation.

Experience may already have told you that most low-yielders are not qualitatively unique; if so, 3a will have to be perfected before 3b is worth much.

Finally, <u>after</u> time study, I notice how <u>few</u> broths are rejected as being familiar. Unless your criteria at this stage are quite reliable, might it not be better to omit characterization tests at this stage, or note them as only advisory to Biocham, and rely on the chromatography? If you do continue the prelim. charact., wouldn't your tentative conclusion simplify the later extraction steps enough to make it worthwhile to do a more rigorous analysis? Aren't you taking an undue chance, in relation to the cost, of missing a good find? I am not much impressed by the deep agar column, by itself, as a disagnostic tool, since an unknown new substance might very well mimic the others. The same for cross-resistance, especially with broad spectrum antibiotics.

Yours sincerely,

Joshua Lederbegg