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ESCUELA DE MEDICINA

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*Rotman*

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Dear Josh:

Thanks for your letter and a bunch of reprints you sent me. It seems we have finally got rid of that ms because I haven't heard from the Editor and Bernie told me he gave it his blessings although he still thinks it is hard to read.

Perhaps my letter was misleading in the sense that I never reacted badly to Bernie's criticism. I think that practically all of them were very justified. Of course, it bothered me to have to correct the ms (I hate all office work, you know), but it improved quite a lot thanks to Bernie in spite of the fact that he still doesn't understand my way of conducting the research.

I don't know if you read the final copy of the ms, but I followed your advise and let the hammer fall on Monod's theories with all its weight.

I plan to continue on the permease story and as a matter of fact I have thought of making active transport my main line of research. It is a rather virgin field in which the microbiologist is just peering in. It's amazing that I have decided this after being sort of drag into the field (I must confess that I never liked too much the activation problem).

Your comment on the Lac<sup>-</sup><sub>1</sub> ability to ferment galactose interest me very much. Of course it will be nice to have the Lac<sup>+</sup>Gal<sup>-</sup><sub>2</sub> which lacks galactokinase, and the Lac<sup>-</sup><sub>1</sub> Gal<sup>-</sup><sub>2</sub>. Please send them to my name with only Borgoño 1470 as the address because it avoids a lot of red tape. Last time it took me six weeks to get the strains from the Post Office.

How are you coming along with the transduction with DNA Chargaff style? Please let me know about it because if it doesn't work it might be still profitable to continue with the labeled DNA experiment. I have ~~now~~ two strains of E. coli thymine-deficient and labeled thymine therefore I could do the experiments rather easily with all the experience I have accumulated with P32 labelling.

About my experiments: A package with radioactive galactose to make C-14 labeled TMG got lost in the mail and therefore I have to wait until a few more weeks to obtain more. In the mean time I have played around with the masking factor of protoplasts. The stability of protoplasts becomes a different problem if one examines the leaking of beta-galactosidase. The problem has not been solved because sucrose, which is the best stabilizing agent inhibits quite a bit the beta-galactosidase at the concentrations used.

Other stabilizing agents reported in literature have failed to stop the release of enzyme from the protoplasts. The protoplasts were prepared either by your method or by Fraser-Mahler's. If you have any comment on this it will be appreciated. If worse comes to worse I plan to compare the accumulation of TMG ~~and~~ the ONPG hydrolysis in protoplasts suspended in buffer with 10 % sucrose.

There is another point related with protoplasts which I would like to submit to your opinion. There is a man here in Chile, a good friend of mine too, who has done a very excellent work on cytology of nerve fibers. I think he has been able to make the thinnest sections known in electron microscopy. A few days ago talking about protoplasts I suggested to him the idea to study sections of protoplasts to compare them with sections of bacteria to see which part, if any, is actually removed by the lysozyme or the penicillin treatment. I was wondering if this work has been done. Do you know anything along this line? Most of the people believe that lysozyme eliminates the cell wall of bacteria when really if the lysozyme dissolves a rigid frame of the cell wall leaving most of its constituents intact the same result would be expected. The electron microscope could discriminate between the two hypotheses if sections are used. The first hypothesis is supported by the fact that lysozyme dissolves preparations of cell walls, but one wonders what is a "preparation of cell walls".

From your ms I found most interesting the formation of protoplasts from DAP requiring cells. Have you another mutants in the biosynthetic pathway of the cell wall? The approach seems quite logical now and I hope it pays up.

Don't worry about my visiting Madison on August. I know better than that. My plans so far are to leave Chile around the end of August and spend two or three months at Bernie's laboratory (if I can finance it). After that period I will make a tour through the Middle West and then back to Chile.

Best regards to you and Esther from Raquel and I,

yours,

*Doris*