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29. 11. 57.

Dear Dr. Lederberg,

Thank you for your letter of 15th Nov. The advertized lactose-mutabile cultures have not arrived yet. Anyhow, I cannot tackle them just now as I am fully occupied with your "K-12" cultures. Yet I intend to examine the mutabile strains later. - I do not think the "K-12"-field is as bare as you seem to suppose. Let me make a confession which I hope you will regard as "confidential" and also quite preliminary. It may

all be a wash out when all the necessary control series will have been done. I do see a number of things which I have only discovered after very prolonged and painstaking searching. This corresponds with your statement that recombinations occur infrequently. Of course, this makes morphological studies very difficult indeed. I am now trying to provoke the cultures to do "it" (whatever it is they are doing) more abundantly. The tricks I apply are the following (1) lowering of the incubation temperature: 30°C, room temp (= 22°C). Further (2) lowering the consistency of the agar to make the medium almost semi-solid. – I am

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always culturing in a moist atmosphere. (3) Now I am trying an atmosphere of 5–10% CO_2. I combine these tricks and try to think of others without changing the chemical composition of the medium. I think the room temp. incubations of soft-medium plates are rather helpfull. I have, though, not seen an increase in the recombinations at the end of the incubation period. Have you ever tried to provoke them to produce more recombinations?

In regard to the minimal medium I should like to ask you

a few questions. I made up a water agar, a glucose solution (10%) and the salt solution 10 times concentrated. I autoclave the salt sol. and water agar and heat the glucose solution in the steamer for 10 mins on three successive days. I then mix the 3 things before pouring the plates to produce the correct concentrations. I use 20 ccm of medium for each plate. Thickness of the agar layer may, of course, also play a role. The organisms grow quite well (microscopically) and show their nuclear structures when Giemsa stained after osmic acid fixation. - HCl etc. are unnecessary. - Please, let me know how you make the medium

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up. Do you use distilled water or tap water? I have been using tap water. The distilled water makes the agar too soft, perhaps because it is always acid here, even directly after boiling. —

If I really should get out a demonstrable story (which is questionable at the present moment) I should like you to check it up thoroughly from the genetical point of view. Perhaps we could then bring out a publication together? My findings may throw some light on the L-cycle too. The beginning of what happens

seems to be in keeping with the L cycle, the remainder seems to be different. – A great complication arises because of the many apparently dying things in the cultures. Many further experiments will have to be done. Yet I am very fascinated by the work and concentrate entirely on it for the time being.

Yours sincerely,

E. Klieneberger-Nobel.

P.S. Would it be allowed to alcalinize the medium and if so with what? How long do you incubate your broth cultures? I give them 11 hours.