February 17, 1951.

Dr. Melvin Cohn,
Institut Pasteur,
Paris, France.

Dear Dr. Cohn:

Thank you very much for your courtesy in sending the MS. copy of your paper with Jacques on the E. coli ML galactosidase. This favor, together with various hints in Monod's recent, but infrequent, letters, and with other less reliable sources, lead me to the inference that I was remiss in not having done the same on the occasion of writing my own paper on the K-12 enzyme. I am sincerely sorry about this omission; if I had known that you had also had a rather parallel contribution in the works, I would certainly have made a point of it. May I however point to two circumstances: a) my own paper was written in considerable haste, just as I was packing for a long trip to California. The MS was mailed as we were leaving town, and proofs were corrected as we were driving back through Nevada or Utah. I was never very satisfied with the ms., especially with the rather sloppy details on lactatic activity, and on the extent of lytic activation, but I have been asked by several people to document the ONPG technique, and it would have taken a great many more months to iron out those details. Another unfortunate consequence of this haste was the loss of a paragraph which literally just slipped out during the typing referring to the Monod-Torrian-Gribetz note on lactase. I did not see the Ann. Inst. Pasteur account until much later. But more important, I had long since written to Monod about most of the details, especially the im-activation effect. Not having heard that you had been working along similar lines, I had no reason to suspect that you would have any special interest in a subject which is not at first sight of much general interest.

As you might suspect, I read your paper with some enthusiasm. I shall look forward especially to seeing the details of your immunochemical analysis, which appears to be approaching a remarkable contribution. Lately, I have been isolating a number of distinct E. coli strains which can be crossed with K-12, and each other. Some of these have distinct somatic antigens, and I had hoped to follow up an immunological analysis to determine whether they might not have serologically distinguishable lactases. It might be possible then to determine whether the determination of enzymatic and serological specificity might be genetically separable. Your demonstration of the cross-reactivity of Aerobacter and two coli lactases is somewhat of a damper, however. At any rate, it is quite apparent that we shall have to lean quite heavily on your exciting work in this area.

A word about the ms. itself. (1st: on p.9 you have a reference to tableII which should read III). One of the ways in which I ran into the Na activation came from the fact that ethanolamine (in -EA-citrate buffer) was markedly inhibitory at quite low concentrations. Is it possible that the tri-ethanolamine is also inhibitory, but that its effect is already maximal (i.e., the competition with H^+ ? already complete) in the concentrations you used in your buffer tests. This could show up by differing sensitivity to K or Na in different TEA buffer concentrations.

One of the most puzzling features of the ionic activation is the nature of its effects on the enzyme kinetics (p. 385 of my paper), which suggested a partial overlapping of the adsorption site with that of the substrate. This picture which I proposed as a merely formal one is given considerable substance by your discovery that the nature of the substrate determines the kind of response. I could not find in your data the means to distinguish between effects on V_m and on K_s. M/600 ONPG is probably saturating for the most K-12 enzyme in certain ionic environments, whereas M/10 lactose certainly will be under most conditions. As part of a general study on this curious phenomenon, one of the graduate students at the enzyme institute is studying the ionic effects on K_s (measured as K_i against ONPG). I will forward his findings as they come up. To date, he has been purifying a batch of enzyme (from 150 g. dry cells - which I understand is quite puny compared to the output of the bactogen), and claims that cold methanol pptn. is a superior technique.

A first reaction might be that duplication such as ours might be unfortunate, but after a little reflection, I am convinced that this is not so, especially because of the diversity of material and approaches used. I would be prepared to discuss any suggestions you might have for planning which lines of work should be followed in the different laboratories, but I think this is probably less important than a frequent and honest exchange of information. I will admit that this is likely to be lopsided, because my facilities are relatively quite limited, and I can spend only a part of my time on enzymological problems (for which reason it is, of course, the genetic aspects which are of closest concern).

Probably, I have already mentioned (to Monod) a mutation leading to the constitutive production of galactosidase. For the present, this seems to be allelic to the Lac_1 mutation (which blocks adaptation to lactose but not to alkyl galactosides). Neolactose (altrose-galactoside) does not provoke lactase, but is attacked readily by it, again separating the adaptive response from the enzymatic attack itself. This property of neolactose was the basis of the original isolation of the constitutive mutation. Cst cells grown on glucose are optimally adapted to ONPG but not to galactose, so that I doubt if it can be a matter of intracellular production of self-adapting galactosides. We are studying now, but unfortunately only slowly, the interaction of Cst with other genotypes, in hybrids and in mixtures. I would give a good deal to have a system in coli K-12 which adapts a little more rapidly so that something could be said of rates of adaptation; from this point of view, Stanier's enzymes are much more satisfactory (aromatic oxidases in Pseudomonas). We found this summer that adaptation could be prevented there with UV, without affecting enzyme activity (his material is not affected by DNP or azide!). The UV-inhibited cells were susceptible to photoreactivation, showing merely that the photoreactivable process is actually, in some senses, a terminal one. We thought that adaptation was more sensitive than viability, but the patent clumping of the cells makes this rather uncertain.

With Deutsch on leave (if only that?), there is a certain hiatus in immunochemistry on this campus. How would you assess the chances of visit back here on your part?- or are you planning a more permanent emigration? Meanwhile, Alain (Bussard) has been showing me and developing a few tricks in paper electrophoresis. Except that this technique readily shows the heterogeneity of K-12 lactase (assayed on the paper by spraying ONPG), nothing has come of it yet.

Sincerely,

Joshua Lederberg

Enc: Reprint (Fink + Lederberg)

PS: Do you want the ms back?