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Dear Dr. Lederberg,

Thank you for your reply to my letter of June 25.

Up to now, I've done two types of work, both of which involved the control of specific protein synthesis. I am essentially interested in antibody formation, but feel that the search for adequate models to describe the control of antibody formation may still be usefully pursued from genetic and biochemical studies with microorganisms. I am interested in knowing how control may be exerted on genetic materials in different states, i. e. integrated or non-integrated, chromosomal or episomal; and how one genetic entity may interact with others in the same cell. Certain intriguing puzzles, such as the reason why lac genes within an entering phage element are not subject to the same control as chromosomal lac, lead one to realize that our present molecular models of control are hardly exhaustive.

In Dr. Coons' laboratory, using the techniques of immunofluorescence, tissue culture and serology, I asked the question--can antibody-producing cells be found in unresponsive mice (whose paralysis had been induced with repeated injections of antigens starting either in adulthood or at birth)? The answer was no. This was true for the adult series, to bovine serum albumin and pneumococcal polysaccharide SII; and for the neonatal series, to BSA or ovalbumin. The neonatal series was inhibited with daily injections of as little as 0.25 $\mu\text{g/g}$, or regularly at 2.5 $\mu\text{g/g}$ and above; whereas the adults were not inhibited at 2.5 $\mu\text{g/g}$, but were at 500 $\mu\text{g/g}$ (only two doses used). No positive cells were visible by fluorescence; no AG-AB complexes could be detected (Farr assays in vitro and in vivo addition of I^{131} -BSA); transfer of unresponsive tissue to normal recipients with or without stimulation at transfer led to no antibody response in the recipients. Perhaps this was all to be expected. Finally, it was shown that the mice recovered "spontaneously" from their unresponsiveness without further stimulation. After recovery, they were in a state of heightened reactivity: an injection of ovalbumin, for example, boosted their antibody level to a point tenfold

above the normal secondary response level of their littermates, who had received no repeated ovalbumin injections.

The concept that these data suggest to me is extremely close to A5: the onset of the antibody response within any cell may be preceded by an unresponsive first stage, in certain ways analogous to repression of enzyme synthesis in bacteria. Accepting as possible A1-A4 (it seems to me that A4 is much more likely now that people think the code is degenerate: the gamma-globulin gene just adds or subtracts a base very readily during replication), we may add the following suggestions:

- Z1--The complete antibody response requires two contacts with antigen.
- Z2--The first of these leads to the production of several stereospecific peptide chains which become enmeshed in the periphery of the cell (L-chains of Edelman *et al.*), converting the cell to a "prepared" cell.
- Z3--Contact with the complete antigen molecule by the prepared cell induces a reaction leading to the production of the rest of the gamma-globulin molecule which presumably could be identical for all antibodies (H-chains).
- Z4--Contact with antigen also stimulates replication and differentiation down the plasma cell path to a dead end in the plasmacyte (mature) which produces its antibody and is then finished. (similar to A7)
- Z5--The first phase of the immune response is inhibited by excess antigen.
- Z6--The repressor sensitive site is located along the genome. (A ribosomal site is unlikely if messenger RNA is a short-lived, continuously synthesized entity in mammals, unless there is some other way of shutting off messenger synthesis; also too many inhibitory molecules of antigen would seem to be necessary, at least one for each ribosome).
- Z7--The unresponsive, tolerant cell when released from inhibition is in the prepared state as in Z2. (In a previous publication, Coons and I called this prepared or memory cell a "Y" cell in an X-Y-Z scheme).
- Z8--The apparently greater susceptibility of neonatal animals to induction of paralysis can be explained by special ad hoc hypotheses. (E.g., the development of an intrinsic adjuvant with age, or the increased accessibility of neonatal cells to penetration by antigen.

A further implication of Z7 is that the cell escaping from paralysis will not produce circulating antibody unless restimulated from without by antigen. The fact that Avriou Mitchison didn't find "spontaneous escape" after paralysis with chick cell antigens is reassuring in this regard, since cellular antigens (in contrast to protein antigens) would be expected to disappear rapidly and not be able to serve as a low level external stimulus to spark plasmacyte differentiation (Z4). Other aspects of this formulation can be tested. For example, does stimulation actually speed escape? What is the course of events immediately upon massive antigen injection; maybe by immunofluorescence or single-cell techniques, pre-paralytic antibody production would be shown. Can one install paralysis in an immune animal? According to your published ideas (unless you include the possibility of repression in mature cells) it should be extremely difficult to install paralysis in an adult because with the mixed population of cells are some which are stabilized to proliferate, etc. and which in this way would be difficult to inhibit unless they recycled through an antigen-sensitive phase. Also, the escape from paralysis should be studied to see whether the first paratype produced corresponds to the epitope in smallest concentration. This should also be expected in the normal antibody response to rather large doses of antigen.

With Luigi Gorini, my work was essentially concerned with the action of small molecules in controlling rates of protein and repressor synthesis. I learned many of the techniques of bacterial physiology and genetics, and of continuous culture. We were able to show, we think, that exogenous arginine has a greater affinity or accessibility for the repressor-forming machinery than the arginine formed endogenously. In Dr. Luria's lab during the next year I hope to learn to deal with a different level of organization, that of macromolecular and supramolecular units (e.g. episomes, transduced genetic material, etc.)

You asked for my specific interests and qualifications, but I'm not sure that this question has been answered. The impression I gave in paragraph three was of preferring epigenetics to genetics, microbial systems to mammalian. Actually, the difference in both these cases appears to be one in the ease of thinking about, and carrying out experiments: it's easier to understand Vivaldi than Webern. But the Weberns can be listened to with enjoyment.

I would appreciate your comments on Z5-7, if you have the time.

Sincerely yours,

Eli Sercarz