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RECOLLECTIONS

The origin of a useful concept—Feedback inhibition

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I would like to recall the circumstance and the setting (both physical and intellectual) that led to the most logically conceived single experiment with the most predictable result ever done in my laboratory. The experiment provided one of the first demonstrations of feedback inhibition, namely that isoleucine was an inhibitor of the first enzymatic step in its pathway, threonine deamination (Fig. 1) (Umbarger, 1956).

The year was 1955; the immediate setting was a hot afternoon of July 19 on the top floor of Building D in what was then the Department of Bacteriology and Immunology at Harvard Medical School. I was sitting in the laboratory of Harold Amos, who had recently returned from a postdoctoral period with Georges Cohen and had picked up an interest in threonine metabolism. We had a mutual interest in the conversion of threonine to α-ketobutyrate, which had only shortly before been confirmed as an essential step in isoleucine biosynthesis by mutant methodology in my laboratory. This was before the days that air conditioners were permitted in our laboratories, but the windows were easily opened. We were probably in Harold's lab rather than mine because his had better cross ventilation.

To appreciate the nature of our considerations, one should recall the state of knowledge at that time among those interested in metabolic pathways and their regulation. Considerable inroads had been made in the analysis of the steps in many biosynthetic pathways, although many uncertainties remained. Already, however, we were thinking about integration of metabolism with the growth process and were wondering how amounts of enzymes present in tissues were regulated. The idea of induced enzyme formation as an unexplained phenomenon was a familiar one. What was later to be operationally defined as "repression" and "derepression" by Henry Vogel (1957) was probably not widely appreciated but was of great interest to some. The examples of which we were aware were the absence or near absence of "methionine synthase" in extracts of *Escherichia coli* cells grown in the presence of methionine as observed by Cohn et al. (1953) and the marked elevation of the valine-, alanine-, α-aminobutyrate-transaminase in cells grown with a restricted supply of valine, which had been found in a cross-country collaboration between Edward Adelberg and me (1953). At that time, the distinction between the effect being due to the end product of the pathway itself or to the absence of a substrate inducer had not been made (but see below).

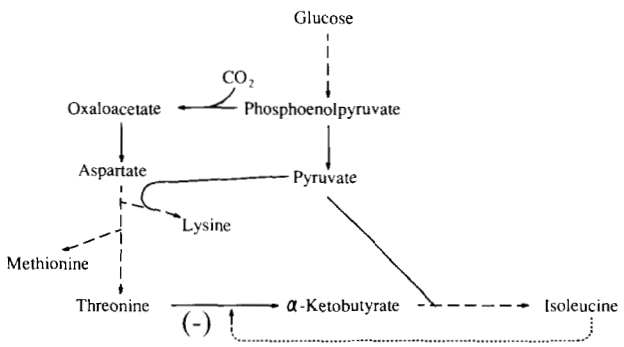

Fig. 1. Route of isoleucine biosynthesis from a simple carbon source (glucose). Broken arrows imply multiple enzymatic steps; solid arrows imply single enzymatic steps. The dotted arrow indicates inhibition (−) by isoleucine of the only step that would account both for quenching of label incorporation into isoleucine from ^{14}C-glucose or $^{14}CO_2$ and for the sparing effect of isoleucine on the threonine requirement of threonine auxotrophs.

The idea that end products did interfere in some way with their own biosynthetic pathways was already quite clear. To my knowledge at the time, the first person to describe such an effect was Joseph Gots, who in 1950 reported at the Atlantic City Federation meetings that the accumulation of 5(4)-amino-4(5)-imidazolecarboxamide by sulfonamide-inhibited *E. coli* was prevented by the

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presence of exogenous purines (Gots, 1950). Gots clearly appreciated the significance of his findings and went on to demonstrate with his students that exogenous purines also prevented precursor accumulation by mutants blocked in purine biosynthesis. In Adelberg's and my collaboration referred to above, we also observed that the accumulation of α-ketoisovalerate, the immediate precursor of valine, by a transaminase B-deficient mutant of *E. coli* was inhibited by exogenous valine. On the same floor at Harvard Medical School, Marcus Brooke (Brooke et al., 1954), working in Boris Magasanik's laboratory, had shown that the accumulation of orotic acid was prevented by exogenous pyrimidine sources, an observation that apparently was being examined further in some detail by Yates and Pardee at about the same time that I was looking at threonine deaminase. Although I don't recall other specific examples that I had in mind at the time, I must have known, through talking with others, that delay in the appearance of intermediates accumulating in culture fluids of mutants until after the required nutrilite had disappeared from the medium was a general phenomenon already observed in several pathways. However, I do not recall being aware of the very good evidence for the control of the tryptophan pathway that had been obtained in the chemostat experiments of Novick and Szillard (1954), although it influenced the thinking of others. Finally, I do not think any of us working with bacterial systems were aware of the experiments of Zacharias Dische, who, during the time of the occupation of France, had shown a control of glucose phosphorylation in red cell lysates by the phosphoglycerates. Had his paper (Dische, 1940) describing those experiments been more widely known, his experiments would certainly have had a seminal effect on the field.

The reports that probably were most influential in my own thinking, and certainly pointed to the generality of the concept of preferential utilization of exogenously supplied nutrilites and a quenching endogenous synthesis, were those from Richard Robert's Biophysics Group at the Department of Terrestrial Magnetism of the Carnegie Institution of Washington and, in particular, the experiments of Philip Abelson. These experiments showed that *E. coli* cells, previously grown in a minimal medium in which their entire spectrum of small molecule building blocks were made from the glucose carbon source and CO_2, fixed from the atmosphere, specifically ceased making any of those compounds when they were supplied in the medium or when precursors of those compounds were supplied (Abelson, 1954). From the nearly complete suppression of endogenous synthesis that occurred, it was clear that preexisting enzymes had to be inhibited or immediately inactivated. Repression of further enzyme synthesis and mere dilution of preexisting enzymes could not have explained their results. Likewise, I was aware of the essentially reverse kind of experiments of Koch et al. (1952) showing that exogenous ^{14}C-labeled purines were preferentially incorporated into DNA over purines formed endogenously.

I must have had a bias toward assuming an internal "induction" as an explanation for the reduced level of biosynthetic enzymes present in cells grown in the presence of the appropriate end products, for Adelberg and I had written: "It [the inhibition of enzyme function by end products] is quite likely a secondary manifestation of the effect of an end product blocking its own [sic] biosynthesis" (Adelberg & Umbarger, 1953). (I shudder today at the contradiction in that sentence, since anything already made cannot prevent its own formation. It was written, however, before Bernard Davis came to Harvard, and I hadn't yet come under the influence of his keen sensitivity to word usage.) However, in special cases, that idea is correct, and the isomeroreductase in the isoleucine and valine pathway is induced by its substrates, and its repression by valine is due to valine inhibiting the formation of the acetohydroxy acids in the K-12 strain of *E. coli*.

It was with that kind of awareness that I reasoned with Harold Amos that, one, the quenching of isoleucine synthesis from the glucose carbon source had to be an immediate effect (i.e., by the inhibition or inactivation of some enzyme in the pathway), but, two, in order to account for the sparing effect of isoleucine on the threonine requirement of threonine mutants, only an inhibition of threonine deamination could account for the results. Boris Magasanik, on whom all of the younger people in the department tested new ideas, was out of the country at the time, but this was such a simple idea that it could be tested over the following 45 minutes.

Over in my lab, Barbara Brown had been comparing several extracts of *E. coli* cells that had been treated in several ways to explore the conditions that lead to loss of threonine deaminase activity. She was getting ready to put the extracts and assay components back into the freezer but still had time for a quick standard assay procedure, which we modified to include a pair of tubes containing L-isoleucine at an uncertain concentration, perhaps 20 mM (if so, it was real overkill). The result, of course, was a 99% inhibition of threonine deamination by that extract, and setting up a protocol for a more precise experiment on the following day.

Over the next few weeks, Barbara Brown and I tried to define more clearly the isoleucine–threonine interactions, because I wanted to submit a short note to *Science*. One troublesome aspect was our inability to obtain nice, expected Michaelis–Menten kinetics. Our kinetic curves were sigmoid, both with respect to substrate binding and with respect to inhibitor binding. Plotting activity or percent inhibition against the square of the substrate or inhibitor concentration gave much prettier curves. If we had used a pH 8.5 or pH 9 buffer, the kinetics would have been "normal." At that pH, however, the enzyme would have been almost resistant to isoleucine inhibition.

I also knew the enzyme was inactive whenever I used Tris-HCl buffer suitable for the higher pH (I didn't know it was because of its monovalent cation requirement). So, we were fortunate that the right conditions were chosen.

Shortly after submitting my manuscript to *Science*, Jacques Monod came through Boston on his way to an international symposium at the Henry Ford Hospital. Having met Boris Magasanik earlier, Monod stopped by to visit Boris in our department. Although I saw him at that time, I did not get to meet or talk with him. Boris, however, did show him my manuscript, and Monod asked to have a copy sent to him after it had been accepted. Clearly, Monod was interested. When the proceedings of that symposium appeared, it was clear that, during one of the discussion periods, Art Pardee had reported on his and Yates' discovery of the sensitivity of the *E. coli* aspartate transcarbamylase to CTP. Probably Monod never told Art about my paper, and of course I did not know of his work until his paper with Yates appeared in the *Journal of Biological Chemistry* (Yates & Pardee, 1956). Monod may have been the only person in the world who knew of our independent and essentially simultaneous observations.

Those were before the days of Xerox or even Thermofax, so that copies meant waiting hat in hand for *the* departmental secretary to type out another. But on February 3, 1956, I did send Monod the copy he requested and expressed my disappointment in not being introduced to him at the time of his Boston visit.

I didn't hear from Monod again until June 1959, when he wrote asking me whether I had any plans to study the acetolactate-forming system, which was the valine-sensitive enzyme and presumably catalyzed the corresponding step in isoleucine biosynthesis. He referred to a very bright young student (what an understatement, as it turned out) working with him and Jacob, by the name of Jean-Pierre Changeux. They wanted to initiate a genetic and biochemical analysis by examining "de-repressed and de-inhibited mutants." He was to keep me fully informed of his progress. At the time I was more interested in the next step in the pathway, although I was still interested in the unknown cofactor required by the acetohydroxy-acid synthase. Thus, I had no qualms about saying go ahead and that I would look forward to hearing of the student's progress.

I heard no more until the time of the Cold Spring Harbor symposium of 1961, when we invited Monod to give the final lecture. When he accepted, he asked to have Changeux put on the program to talk not on the acetolactate-forming system but on threonine deaminase. The rest of the story is, of course, well-known history. Monod's final lecture was not the summary session we had anticipated but the unveiling (to many of us, at least) of the allosteric concept (Monod & Jacob, 1961). Monod had a broader visualization of the concept than I had developed, and threonine deaminase as well as my largely ignored 1956 *Science* paper were put on the map.[1] His interest in the enzyme provided the impetus for the term "allosteric enzymes" and led eventually to the idea of concerted transitions between two states in multimeric proteins as the basis for cooperative effects in regulatory proteins. Although I have yet to see good evidence that the concerted model accounts for regulation of threonine deaminase, the concept is a convenient one to distinguish between the inhibitor-bound and substrate-bound forms of the enzyme.

Although the main general message in my *Science* paper was the principle that we should expect the initial irreversible step in a biosynthetic pathway to be inhibited by the end product of the pathway, there was another important message inherent in the available information. This is that an enzyme, selected for a very specialized role in metabolism with either its formation or its activity tightly controlled by a specific set of factors, is not suitable for catalysis of that very same reaction to serve another metabolic function in the cell. For that other role, a different enzyme is required. The specific example I had in mind was the degradative threonine deaminase described earlier by Wood and Gunsalus (1949) versus the biosynthetic enzyme required for isoleucine biosynthesis. Although it was possible to contrive conditions whereby the degradative enzyme gave rise to α-ketobutyrate needed for isoleucine biosynthesis, it could not normally fulfill that function. In other words, a highly specialized adaptation to one role mitigates against the flexibility to serve in another. It is as true for a protein in *E. coli* as it is for the snail darter in the Tellico River.

That concept is a lesson that I learned only after appreciating the significance of the two threonine deaminases. As a result, when Murray Strassman and Sidney Weinhouse had earlier proposed acetolactate as an intermediate in valine biosynthesis (Strassman et al., 1953), I pooh-poohed the idea, since I, like every other bacteriologist who had learned about the "IMViC" test "knew" that *E. coli*, in contrast to *Aerobacter aerogenes*, could not form acetolactate (an example of how incomplete knowledge can be more harmful than ignorance). It was not until Barbara Brown and I found acetolactate accumulating in *E. coli* mutants that I became a believer. It was then, with the help of Yeheskel Halpern, that we extended the lesson of two specialized threonine deaminases to the occurrence of the degradative acetolactate synthase as well as the biosynthetic synthase in *A. (Klebsiella) aerogenes* (Halpern & Umbarger, 1959).

[1] There is a graphical mistake in the paper that should be corrected for any readers that might consult it at this time. A plot is given of the v_0/v_1 (ratio of uninhibited to inhibited velocity) against [isoleucine]^2. By definition, the y-intercept would be 1.0. It was not until 30 years later, when some friends conspired to commemorate the appearance of that article at the Purdue Biochemistry Retreat and presented me with a paperweight with a reproduction of that figure, that I "saw" for the first time my old mistake, an errant decimal point giving an impossible y-intercept of 0.1, just as it appeared in the paper!

Actually, because I had no mutants blocked in acetolactate or acetohydroxybutyrate formation, I had used the sensitivity of the enzyme to valine to support the case for acetolactate being an intermediate in valine biosynthesis. The reason for there being no such mutants was that the *E. coli* K-12 strain had two enzymes catalyzing the step, and most other *E. coli* strains had a third. It was not until 1974 when Maurizio Iaccarino's group in Naples went through a series of ingenious single mutagenic steps that the isozymic nature of these enzymes was deduced (Guardiola et al., 1974).

In the years since Monod drew everyone's attention to the importance of such proteins as threonine deaminase and aspartate transcarbamylase, the finding of cooperative effects in proteins that respond to a regulatory signal has become commonplace and even the expected behavior. It is interesting that in the paper by Yates and Pardee on aspartate transcarbamylase (Yates & Pardee, 1956) a pH had been chosen (6.5) that almost obliterated the cooperativity shown by the enzyme at higher pH values. We now well appreciate what really had been known since the oxygen-carrying properties of hemoglobin were contrasted with those of myoglobin, namely that the most effective regulatory response is one that occurs over a relatively narrow range of substrate or regulatory effector concentrations. At an earlier time, such "abnormal" kinetics in enzymes were viewed with alarm.

Threonine deaminase has trailed in its impact behind aspartate transcarbamylase and some other regulatory proteins, not only because it has a rather small role in metabolism but also because as yet the tertiary and quaternary structure of the protein has not been described. The molecular pattern found in aspartate transcarbamylase, in which the regulatory and catalytic domains are found on separate subunits, is not the pattern found in threonine deaminase and most other regulatory proteins. Comparison of the biosynthetic threonine deaminase of plants, bacteria, and yeast with the biodegradative enzyme of *E. coli* and the threonine (and serine) deaminases of animals reveals that the latter lack an extensive region toward the C-terminal end that presumably constitutes a covalently linked regulatory domain. It might therefore seem that threonine deaminase, had a three-dimensional structure been available, would have been a better paradigm for allosteric enzymes than is aspartate transcarbamylase. It is a fundamental question, however, whether the interactions between inhibitor sites and substrate sites or between regulatory and catalytic domains are essentially the same or different when the domains are covalently linked instead of being on separate subunits. It is a question that can be answered only by future structure–function analyses being done with the background of a good three-dimensional model of an enzyme such as threonine deaminase.

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