Orders of Magnitude

We shall assume the K(M) have values of the order of magnitude $K(M) = 10^{-6}$ mol./liter, and further we shall assume that we have

$$10^{-3} > \frac{K(M)}{K_{rep}} > 10^{-5} \qquad (1)$$

We estimate that in the wild type bacterium growing in minimal medium the concentration of the metabolite, M, lies between 1/10th K/M and 10 K/M

$$\frac{1}{10} K/M < [M] < 10\,K/M \qquad (2)$$

If the molecular weight of the metabolite is of the order of magnitude of 100 gram, this would then correspond to an order of magnitude of 1 gamma per xxxxx and 100 gamma per liter. We further assume that

$$10^{3} < \frac{K(M)}{K_{rep}} < 10^{5} \qquad (3)$$

From these assumptions and estimates, it follows that if the wild type bacterium grows in minimal medium and if the concentration of Kep(n) is about 10 times lower than the concentration of the metabolite, M, then the rate of enzyme production in the bacterium is suppressed about 10^4 fold, provided that $\frac{M}{K_M} = +$

We may now examine what happens if we raise the concentration of M within the wild type bacterium that grows in minimal medium by adding the metabolite, for instance, a pyrimidine or an amino acid, such as arginine, to the medium in which the bacterium grows. (a) Let us deal first with the case

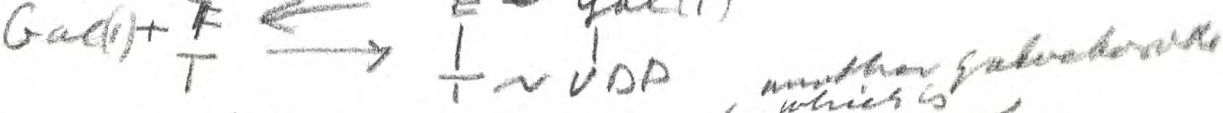
The theory here presented predicts, for instance in the case of a metabolic pathway leading to an amino acid (M = AA), that in certain specific circumstances a precursor of the amino acid M, for instance, A(-1), may induce the formation of the enzyme, E. The conditions under which this may happen are as follows: Let us consider a mutant which lacks, for instance, enzyme E(-2), and which therefore requires arginine as a growth factor. If this mutant is grown in a chemistat with arginine as a limiting growth factor slowly, then, as the equations show, the rate of the production of the enzyme E is increased (for very slow growth in the Chemostat, the enzyme increases by at least a factor of 2) in the worst case when this particular amino acid limited the growth rate even in the wild type growing in minimal medium. In general one may expect a much greater increase. The theory here presented predicts that when the same mutant is grown with arginine as a limiting growth factor rather fast just a few per cent lower than the minimal growth rate of the mutant in the presence of large amounts of arginine, then the precursors of the amino acid, AA-1 or AA-2 will induce the synthesis of the enzyme E. This prediction is based on the assumption that a coupling enzyme, E_o, cannot handle these precursors and that therefore no repressor is formed from the precursors by the bacterium. A precursor of the metabolite, M, will however act as

Appendix

Recently /Milton Weiner ~~recently~~ paved the way to ~~the~~ unraveling of the mystery of the induction of β-galactosidase by introducing the notion that this enzyme is formed in ~~bacteria which had formed it~~, not because the enzyme is capable of splitting lactose, but rather because this enzyme is instrumental in transferring galactose residues to the polysaccharide that forms part of the cell wall. He suggested in particular that the substrate of the enzyme ~~is~~ might be gal-1-P ~~or~~ and more likely is UDP-Gal. I shall here assume, rightly or wrongly, for the sake of argument that there are two enzymes involved. The enzyme, β-galactosidase, or E(2), which transforms UDPGal into a derivative UPD Gal(1) and another enzyme, E(X), which transferes UDP Gal(1) to the polysaccharide of the cell wall.

UDPGal $\xrightarrow{E(2)}$ UPD GAL(1) $\xrightarrow{E(X)}$

I assume that UPD Gal(1) is a repressor which ~~The compound~~ represses the formation of ~~the enzyme β-galactosidase~~ E(2) by reversibly forming the complex



The repressor is a galactoside. In the presence of its chemical analogue, another galactoside which is ~~the inducer~~ TMG, the enzyme template complex is protected if the TMG is present in sufficiently high concentrations inside the cell. ~~TMG is thus~~ a real inducer because, according to our assumption, it ~~covers~~ protects the enzyme template complex. The compound UPD Gal is a precursor of the repressor. ~~If cells are grown in glucose and if, thereby, the level of this precursor is increased, the production of the enzyme is repressed at low concentrations of TMG.~~

Because the rate of the production of the enzyme, Z, increases faster than linearly with increasing TMG concentration, we must assume that the inducer, TMG, might also act by competing with UPDGal for ~~the enzyme~~ 2 and thereby reducing the rate of production of the ~~chief~~ repressor" UDPGal(1) . It is further possible that TMG also competes with the compound UDP Gal for enzyme 2 and thereby slows down the production of UDP Gal which, ~~according to our scheme~~ is a precursor of the ~~chief~~ "repressor". Why does TPG thiophenyl-β-galactoside inhibit the formation of the enzyme at moderate concentrations of the inducer, TMG, within the cell? There are two possibilities. TPG may compete with the chief repressor for the enzyme E_X, which eliminates this repressor by transferring it to the cell wall, and in addition TPG might compete with UDP Gal for enzyme 3 and thereby prevent this compound from being elimated by going over to Gal-1-P, which might conceivably be transferred in some unknown way to the polysaccharide of the cell wall.

As stated before, the constitutive mutans are likely to mutants which have a high equilibrium constant for the reaction in which they combine with the enzyme template complex. Consequently, we might expect that in constitutive mutants, which do not make enzyme at the full rate, β-galactosides, which have a higher equilibrium constant than TMG for combination with the enzyme template complex and which therefore do not induce the wild type, may nevertheless successfully compete in such a mutant with the repressor. Perhaps the fastest way to unravel in detail the phenomenon of β-galactosidase induction would consist in studying the induction of this system when the cells grow in the absence of any other carbon source on galactose.

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A chemical analogue of a metabolite can enhance the synthesis of an enzyme not only by competing for the enzyme-template complex with a repressor but also by competing with a substrate for an enzyme that makes the repressor (or makes a precursor of the repressor). This mode of action of an inducer (which, incidentally, need not be a real inducer in the terms of our definition) was first proposed by Werner Maas (oral communication; breakfast, April 26, 1957), and helped me greatly to clarify my thinking on the general subject of enzyme induction. Indeed, we must invoke this mode of action of an inducer if we want to explain, for instance, why the rate of synthesis of an enzyme such as β-galactose rises faster than linearly with the concentration of an inducer like TMG. By its competition with the repressor for an enzyme template-complex, gives never a faster than linear rise for the rate of enzyme synthesis with the concentration of the inducer.

By extending Maas' idea, we may now further say that an inhibitor of enzyme induction such as, for instance, TPG must be a chemical analogue of the substrate of an enzyme that transforms the repressor into an inert compound, and that it must inhibit such an enzyme.

Better phrasing?

By way of extension of Maas's suggestion we may now further say a chemical compound (such as TPG for instance, which specifically inhibits) can induce the formation of an enzyme only if it is a chemical analogue of a substrate of an enzyme that is involved in converting the repressor into a nuclear repressor (or a non repressor), and of the