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From enzymatic adaptation to allosteric transitions

Nobel Lecture, December 11, 1965

One day, almost exactly 25 years ago - it was at the beginning of the bleak winter of 1940 - I entered André Lwoff's office at the Pasteur Institute. I wanted to discuss with him some of the rather surprising observations I had recently made.

I was working then at the old Sorbonne, in an ancient laboratory that opened on a gallery full of stuffed monkeys. Demobilized in August in the Free Zone after the disaster of 1940, I had succeeded in locating my family living in the Northern Zone and had resumed my work with desperate eagerness. I interrupted work from time to time only to help circulate the first clandestine tracts. I wanted to complete as quickly as possible my doctoral dissertation, which, under the strongly biometric influence of Georges Teissier, I had devoted to the study of the kinetics of bacterial growth. Having determined the constants of growth in the presence of different carbohydrates, it occurred to me that it would be interesting to determine the same constants in paired mixtures of carbohydrates. From the first experiment on, I noticed that, whereas the growth was kinetically normal in the presence of certain mixtures (that is, it exhibited a single exponential phase), two complete growth cycles could be observed in other carbohydrate mixtures, these cycles consisting of two exponential phases separated by a-complete cessation of growth (Fig.1).

Lwoff, after considering this strange result for a moment, said to me, "That could have something to do with enzyme adaptation."

"Enzyme adaptation? Never heard of it!" I said.

Lwoff's only reply was to give me a copy of the then recent work of Marjorie Stephenson, in which a chapter summarized with great insight the still few studies concerning this phenomenon, which had been discovered by Duclaux at the end of the last century. Studied by Dienert and by Went as early as 1901 and then by Euler and Josephson, it was more or less rediscovered by Karström, who should be credited with giving it a name and attracting attention to its existence. Marjorie Stephenson and her students Yudkin and

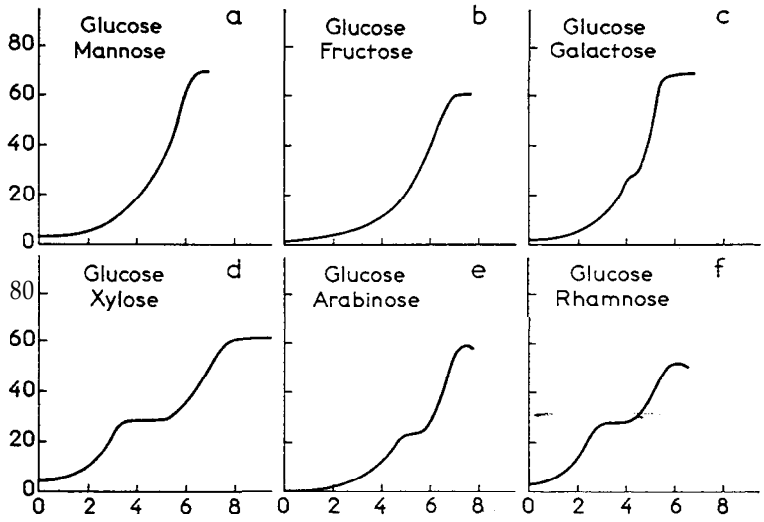

Fig.1. Growth of *Esherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[50].

Gale had published several papers on this subject before 1940. [See ref. 1 for a bibliography of papers published prior to 1940]

Lwoff's intuition was correct. The phenomenon of "diauxy" that I had discovered was indeed closely related to enzyme adaptation, as my experiments, included in the second part of my doctoral dissertation, soon convinced me. It was actually a case of the "glucose effect" discovered by Dienert as early as 1900, today better known as "catabolic repression" from the studies of Magasanik[2].

The die was cast. Since that day in December 1940, all my scientific activity has been devoted to the study of this phenomenon. During the Occupation, working, at times secretly, in Lwoff's laboratory, where I was warmly received, I succeeded in carrying out some experiments that were very significant for me. I proved, for example, that agents that uncouple oxidative phosphorylation, such as 2,4-dinitrophenol, completely inhibit adaptation to lactose or other carbohydrates[3]. This suggested that "adaptation" implied an expenditure of chemical potential and therefore probably involved the true synthesis of an enzyme. With Alice Audureau, I sought to discover the still quite obscure relations between this phenomenon and the one Massini, Lewis, and others had discovered: the appearance and selection of "spontaneous" mutants (see ref.1). Using a strain of *Escherichia coli mutabile* (to which we had given the initials ML because it had been isolated from Andre Lwoff's intes-

tinal tract), we showed that an apparently spontaneous mutation was allowing these originally "lactose-negative" bacteria to become "lactose-positive". However, we proved that the original strain *(Lac-)* and the mutant strain *(Lac+)* did not differ from each other by the presence of a specific enzyme system, but rather by the ability to produce this system in the presence of lactose. In other words, the mutation affected a truly genetic property that became evident only in the presence of lactose[4].

There was nothing new about this; geneticists had known for a long time that certain genotypes are not always expressed. However, this mutation involved the selective control of an enzyme by a gene, and the conditions necessary for its expression seemed directly linked to the chemical activity of the system. This relation fascinated me. Influenced by my friendship with and admiration for Louis Rapkine, whom I visited frequently and at length in his laboratory, I had been tempted, even though I was poorly prepared, to study elementary biochemical mechanisms, that is, enzymology. But under the influence of another friend whom I admired, Boris Ephrussi, I was equally tempted by genetics. Thanks to him and to the Rockefeller Foundation, I had had an opportunity some years previously to visit Morgan's laboratory at the California Institute of Technology. This was a revelation for me - a revelation of genetics, at that time practically unknown in France; a revelation of what a group ofscientists could be like when engaged in creative activity and sharing in a constant exchange of ideas, bold speculations, and strong criticisms. It was a revelation of personalities of great stature, such as George Beadle, Sterling Emerson, Bridges, Sturtevant, Jack Schultz, and Ephrussi, all of whom were then working in Morgan's department. Upon my return to France, I had again taken up the study of bacterial growth. But my mind remained full of the concepts of genetics and I was confident of its ability to analyze and convinced that one day these ideas would be applied to bacteria.

«*Discovery*» *of Bacterial Genetics*

Toward the end of the war, while still in the army, I discovered in an American army bookmobile several miscellaneous issues of Genetics, one containing the beautiful paper in which Luria and Delbrücks demonstrated for the first time rigorously, the spontaneous nature of certain bacterial mutants. I think I have never read a scientific article with such enthusiasm; for me, bacterial genetics was established. Several months later, I also "discovered" the paper

by Avery, MacLeod, and McCarty[6] - another fundamental revelation. From then on I read avidly the first publications by the "phage-church", and when I entered Lwoff's department at the Pasteur Institute in 1945, I was tempted to abandon enzyme adaptation in order to join the church myself and work with bacteriophage. In 1946 I attended the memorable symposium at Cold Spring Harbor where Delbrück and Bailey, and Hershey, revealed their discovery of virus recombination at the same time that Lederberg and Tatum announced their discovery of bacterial sexuality[7]. In 1947 I was invited to the Growth Symposium to present a report[1] on enzyme adaptation, which had begun to arouse the interest of embryologists as well as of geneticists. Preparation of this report was to be decisive for me. In reviewing all the literature, including my own, it became clear to me that this remarkable phenomenon was almost entirely shrouded in mystery. On the other hand, by its regularity, its specificity, and by the molecular-level interaction it exhibited between a genetic determinant and a chemical determinant, it seemed of such interest and of a significance so profound that there was no longer any question as to whether I should pursue its study. But I also saw that it would be necessary to make a clean sweep and start all over again from the beginning.

The central problem posed was that of the respective roles of the inducing substrate and of the specific gene (or genes) in the formation and the structure of the enzyme. In order to understand how this problem was considered in 1946, it would be well to remember that at that time the structure of DNA was not known, little was known about the structure of proteins, and nothing was known of their biosynthesis. It was necessary to resolve the following question: Does the inducer effect total synthesis of a new protein molecule from its precursors, or is it rather a matter of the activation, conversion, or "remodeling" of one or more precursors?

This required first of all that the systems to be studied be carefully chosen and defined. With Madeleine Jolit and Anne-Marie Torriani, we isolated β-galactosidase, then the amylomaltase of *Escherichia coli*[8]. *Our* work was advanced greatly by the valuable collaboration of Melvin Cohn, an excellent immunologist, who knew better than I the chemistry of proteins. He knew, for example, how to operate that marvelous apparatus that had intimidated me, the "Tiselius"[9]. With Anne-Marie Torriani, he characterized β-galactosidase as an antigen[10]. Being familiar with the system, we could now study with precision the kinetics of its formation. A detailed study of the kinetics carried out in collaboration with Alvin Pappenheimer and Germaine Cohen-Bazire[11] strongly suggested that the inducing effect of the substrate entailed

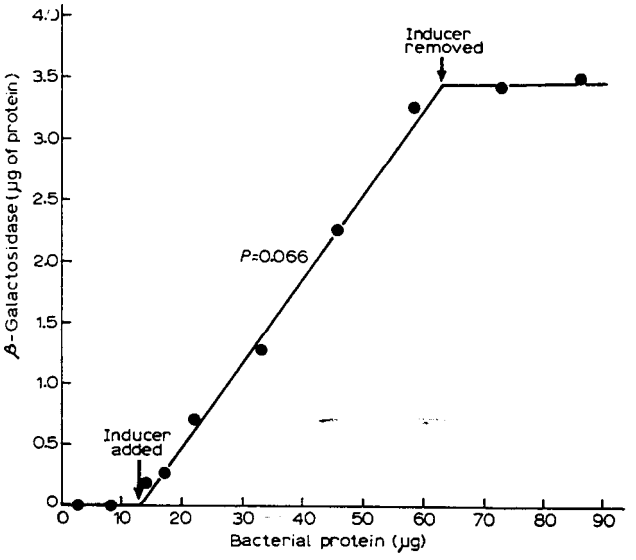

Fig.2. Induced biosynthesis of β-galactosidase in *Escherichia coli.* The increase in enzyme activity is expressed not as a function of time but as a function of the concomitant growth of bacterial proteins. The slope of the resulting curve (P) indicates the differential rate of synthesis[11].

total biosynthesis of the protein from amino acids (Fig. 2). This interpretation seemed surprising enough at that time, but from the first, I must say, it won my firm belief. There is in science, however, quite a gap between belief and certainty. But would one ever have the patience to wait and to establish the certainty if the inner conviction were not already there?

We were to establish certainty a little later, thanks to some experiments with isotopic tracers done by Hogness, Cohn, and myself[12]. To tell the truth, the results of these labeling experiments were even more surprising in view of the ideas then current on the biosynthesis ofproteins and their state within the cell. The work of Schoenheimer[13] had actually persuaded most biochemists that in an organism proteins are in herently in a "dynamic state", each molecule being perpetually destroyed and reconstructed by exchange of amino acid residues. Our experiments, however, showed that β-galactosidase is entirely stable in *vivo,* as are other bacterial proteins, under conditions of normal growth. They did not, of course, contradict the results of Schoenheimer, but very seriously questioned their interpretation and the dogma of the "dynamic state".

Be that as it may, these conclusions were invaluable to us. We knew, thenceforth, that "enzyme adaptation" actually corresponds to the total biosyn-

thesis of astable molecule and that, consequently, the increase of enzyme activity in the course of induction is an authentic measure of the synthesis of the specific protein.

These results took on even more significance as our system became more accessible to experiment. With Germaine Cohen-Bazire and Melvin Cohn [14,15], I was able to continue the systematic examination of a question I had repeatedly encountered: the correlations between the specificity of action of an inducible enzyme and the specificity of its induction. Pollock's pertinent observations on the induction of penicillinase by penicillin[16] made it necessary to consider this problem in a new way. We conducted a study of a large number of galactosides or their derivatives, comparing their properties as inducers, substrates, or as antagonists of the substrates of the enzyme, once more reaching a quite surprising conclusion, namely, that inductive ability is by no means a prerogative of the substrates of the enzyme, or even of the substances capable

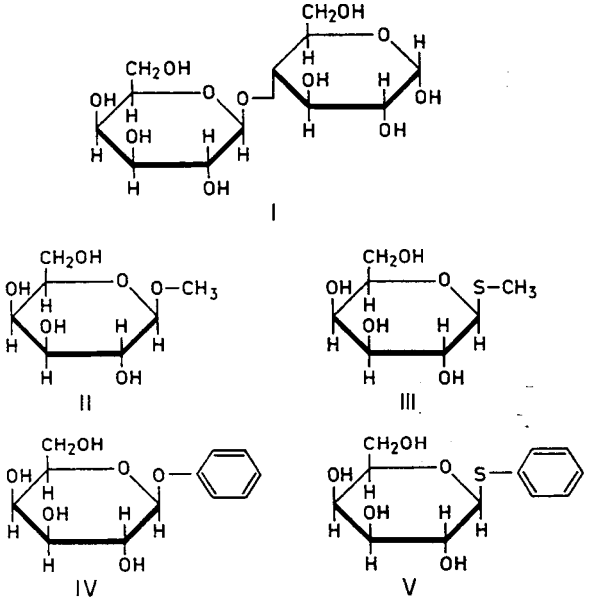

Fig. 3. Comparison of various β-galactosides as substrates and as inducers of β-galactosidase. I, Lactose: substrate of the enzyme, but deprived of inductive activity. II, Methyl-β-D-galactoside: low-affinity substrate effective inducer. III, Methyl-β-D-thiogalactoside: not hydrolyzable by the enzyme, but a powerful inducer. IV, Phenyl- β-D-galactoside : excellent enzyme substrate, high affinity, no inductive ability. V, Phenyl- β-D- thiogalactoside : no activity either as a substrate or as an inducer, but capable of acting as an antagonist of the inducer.

of forming the most stable complexes with it. For example, certain thiogalactosides, not hydrolyzed by the enzyme or used metabolically, appeared to be very powerful inducers. Certain substrates, on the other hand, were not inducers. The conclusion became obvious that the inducer did not act (as frequently assumed) either as a substrate or through combination with preformed active enzyme, but rather at the level of another specific cellular constituent that would one day have to be identified (Fig. 3).

Generalized Induction

In the course of this work, we observed-a fact that seemed very significant. a certain compound, **phenyl-β-D-thiogalactoside,** devoid of inductive capacity, proved capable of counteracting the action of an effective inducer, such as methyl-β-D-thiogalactoside. This suggested the possibility of utilizing such "anti-induction" effects to prove a theory that we called, somewhat ambitiously, "generalized induction". From the very beginning of my research, I had been preoccupied with the problem posed by the existence, together with inducible enzymes, of "constitutive" systems; in other words (according to the then current definition), systems synthesized in the absence of any substrate or exogenous inducer, as is the case, of course, with all the enzymes of intermediate and biosynthetic metabolism. It did not seem unreasonable to suppose that the synthesis of these enzymes was controlled by their endogenous substrate, which would imply that the mechanism of induction is in reality universal. We were encouraged in this hypothesis by the work of Roger Stanier on the supposedly sequential induction of systems attacking phenolic compounds in *Pseudomonas.*

I sought, therefore, along with Germaine Cohen-Bazire, to prove that the biosynthesis of a typically "constitutive" enzyme (according to the ideas of the time), tryptophan synthetase, could be inhibited by an analogue of the presumed substrate. The reaction product seemed a good candidate for an analogue of the substrate, and we were soon able to prove that tryptophan and 5-methyltryptophan are powerful inhibitors of the biosynthesis of the enzyme. This was the first known example of a "repressible" system - discovered, it turned out, as proof of a false hypothesis[17].

I did not have, I must say, complete confidence in the ambitious theory of "generalized" induction, which soon encountered various difficulties. I was, however, encouraged by an interesting observation made by Vogel and

Davis[18] concerning another enzyme, acetylomithinase, involved in the formation of arginine. Using a mutant requiring arginine or *N*-acetylomithine, Vogel and Davis found that, when the bacteria are cultivated in the presence of arginine, they do not produce acetylomithinase, whereas when they are cultivated in the presence of N-acetylomithine, acetylomithinase is synthesized. Hence these authors concluded that this enzyme must be induced by its substrate, N-acetylomithine. When Henry Vogel was passing through Paris, I drew his attention to the fact that their very interesting observations could just as well be explained as resulting from an inhibitory effect of arginine as from an inductive effect of acetylomithine. In order to resolve this problem, it was necessary to study the biosynthesis of the enzyme in a mixture of the two metabolites. The experiment proved that it is indeed a question of an inhibiting effect rather than an inductive effect. Vogel, quite rightly, proposed the term "repression" to designate this effect and thus established "repressible" systems alongside of "inducible" systems. Later on, thanks especially to the studies of Maas, Gorini, Pardee, Magasanik, Cohen, Ames, and many others (see ref.19 for literature), the field of repressible systems was considerably extended; it is now generally accepted that practically all bacterial biosynthetic systems are controlled by such mechanisms.

Nevertheless, I remained faithful to the study of the β-galactosidase of *Escherichia coli,* knowing well that we were far from having exhausted the resources of this system. During the years spent in establishing the biochemical nature of the phenomenon, I had been able only partially to approach the question of its genetic control - enough, however, to convince me that it was extremely specific and that it justified the idea that Beadle and Tatum's postulate, "one gene-one enzyme", was applicable to inducible and degradative enzymes as well as to the enzymes of biosynthesis, which the Stanford school had principally studied. These conclusions led me to abandon an idea I had adopted as a working hypothesis - that is, that many different inducible enzymes may result from the "conversion" of a single precursor whose synthesis is controlled by a single gene; this hypothesis was also contradicted by the results of our experiments with tracers.

But genetic analysis once more encountered grave difficulties. First, the low frequency ofrecombination, in the systems ofconjugation known at that time, did not permit fine genetic analysis. Another difficulty holding us back was the existence of some mysterious phenotypes; certain mutants ("cryptic"), incapable of metabolizing the galactosides, nevertheless appeared capable of synthesizing β-galactosidase. The solution to this problem came to us by

accident while we were looking for something entirely different. In 1954, when the chairmanship of the new Department of Cellular Biochemistry had just been bestowed upon me, Georges Cohen joined us, and I suggested to him, and simultaneously to Howard Rickenberg, to make use of the properties of thiogalactosides as gratuitous inducers in attempting to study their fate in inducible bacteria, employing a thiogalactoside labeled with carbon-14. We noted that the radioactivity associated with the galactoside accumulated rapidly in wild-type induced bacteria, but not in the so-called cryptic mutants. Neither did the radioactivity accumulate in wild-type bacteria not previously induced. The capacity for accumulation depended, therefore, on an inducible factor. Study of the kinetics, of the specificity of action, and of the specificity of induction of this system, as well as the comparison of various mutants, led us to the conclusion that the element responsible for this accumulation could only be a specific protein whose synthesis, governed by a gene (*y*) distinct from that of galactosidase *(z),* was induced by the galactosides at the same time as the synthesis of the enzyme. To this protein we gave the name "galactoside permease"[20,21] (Fig.4).

The very existence of a specific protein responsible for the permeation and accumulation of galactosides was occasionally put in doubt because the evidence for it was based entirely on observations *in vivo.* Some of the researchers who did not really doubt its existence still reproached me from time to time for giving a name to a protein when it had not been isolated. This attitude reminded me of that of two traditional English gentlemen who, even if they know each other well by name and by reputation, will not speak to each other before having been formally introduced. On my part, I never for a moment doubted the existence of this protein, for our results could be interpreted in no other way. Nevertheless, I was only too happy to learn, recently, that by a recent series of experiments, Kennedy has identified *in vitro* and isolated the specific inducible protein, galactoside permease[22]. Kennedy was brilliantly successful where we had failed, for we had repeatedly sought to isolate galactoside permease *in vitro.* These efforts of ours, however, were not in vain, since they led Irving Zabin, Adam Kepes, and myself to isolate still another protein, galactoside transacetylase[2 3]. For several weeks we believed that this enzyme was none other than the permease itself. This was an erroneous assumption, and the physiological function of this protein is still totally unknown. It was a profitable discovery, nevertheless, because the transacetylase, determined by a gene belonging to the lactose operon, has been very useful to experimenters, if not to the bacterium itself.

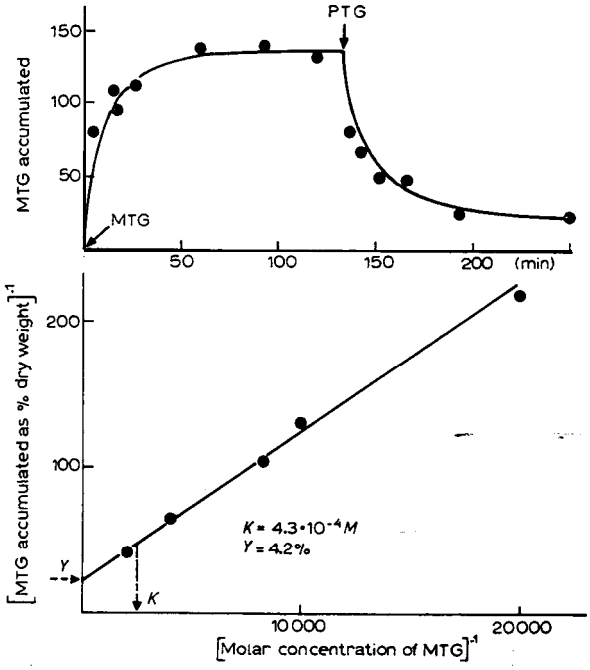

Fig.4. Evidence for the existence of galactoside permease. (Top) Accumulation of labeled methyl-β-D-thiogalactoside (MTG) by a suspension of previously induced bacteria. Displacement of accumulated galactoside(phenyl- β-D-thiogalactoside, PTG) . (Bottom) Accumulation of a galactoside in previously induced bacteria as a function of the concentration of the external galactoside. Inverse coordinates: The constants *K* and Y define, respectively, the constant of apparent dissociation and the constant of apparent activity of the system of accumulation[21].

The study of galactoside permease was to reveal another fact of great significance. Several years earlier, following Lederberg's work, we had isolated some "constitutive" mutants of β- galactosidase, that is, strains in which the enzyme was synthesized in the absence of any galactoside. But we now proved that the constitutive mutation has a pleiotropic effect. In these mutants, galactoside permease as well as galactosidase (and the transacetylase) were indeed simultaneously constitutive, whereas we knew on the other hand that each of the three proteins is controlled by a distinct gene. We then had to admit that a constitutive mutation, although very strongly linked to the loci governing galactosidase, galactoside permease, and transacetylase, had taken place in a gene *(i)* distinct from the other three (*z, y,* and *Ac),* and that the relationship of this gene to the three proteins violated the postulate of Beadle and Tatum.

New Perspectives

These investigations were given new meaning by the perspectives opened to biology around 1955. It was in 1953 that Watson and Crick, on the basis of observations made by Chargaff and Wilkins, proposed their model of the structure of DNA. From the first, in this complementary double sequence, one could see a mechanism for exact replication of the genetic material. Meanwhile, one year earlier, Sanger had described the peptide sequence of insulin, and it was also already known, from the work of Pauling and Itano[24] in particular, that a genetic mutation can cause a limited modification in the structure of a protein. In 1954, Crick and Watson[25] and Gamow[26] proposed the genetic code theory: The primary structure of proteins is determined and defined by the linear sequence of the nucleotides in DNA. Thus the profound logical intuition of Watson and Crick had allowed them to discover a structure that immediately explained, at least in principle, the two essential functions long assigned by geneticists to hereditary factors: to control its own synthesis and to control that of the nongenetic constituents. Molecular biology had been born, and I realized that, like Monsieur Jourdain, I had been doing molecular biology for a long time without knowing it.

More than ten years have elapsed since then, and the ideas whose hatching I recall here were then far from finding a uniformly enthusiastic audience. My conviction, however, had been established long before absolute certainty could be acquired. This certainty exists today, thanks to a succession of discoveries, some of them almost unhoped for, that have enriched our discipline since that time.

Once the physiological relations of galactosidase and galactoside permease were understood, and once it was proved that they depend on two distinct genetic elements while remaining subject to the same induction determinism and to the same constitutive mutations, it became imperative to analyze the corresponding genetic structures. In particular, the expression of these genes and the relations of dominance between their alleles had to be studied in detail.

Precisely at this time, the work of Jacob and Wollman[27] had clarified the mechanism of bacterial conjugation; we knew that this conjugation consists of the injection, without cytoplasmic fusion, of the chromosome of a male bacterium into a female. It was even possible to follow the kinetics of penetration of a given gene. I decided, along with Arthur Pardee and François Jacob, to use these new experimental tools to follow the "expression" of the z^+ and i^+ genes injected into a female carrying mutant alleles of these genes.

This difficult undertaking, carried out successfully thanks to the experimental talent of Arthur Pardee, brought about two remarkable and at least partially unexpected results. First, the *z* gene (which we knew to be the determinant of the structure) is expressed (by the synthesis of β -galactosidase) very fast and at maximum rate from the beginning. I will pass over the development and the consequences of this observation, which was one of the sources of the messenger theory. Second, the inducible allele of the *i* gene is dominant with respect to the constitutive allele, but this dominance is expressed very slowly. Everything seemed to indicate that this gene is responsible for the synthesis of a product that inhibits, or represses, the biosynthesis of the enzyme. This was the reason for designating the product of the gene as a "repressor" and hypothesizing that the inducer acts not by provoking the synthesis of the enzyme but by "inhibiting an inhibitor" of this synthesis[28].

Theory of Double Bluff

Of course I had learned, like any schoolboy, that two negatives are equivalent to a positive statement, and Melvin Cohn and I, without taking it too seriously, debated this logical possibility that we called the "theory of double bluff", recalling the subtle analysis of poker by Edgar Allan Poe.

I see today, however, more clearly than ever, how blind I was in not taking this hypothesis seriously sooner, since several years earlier we had discovered that tryptophan inhibits the synthesis of tryptophan synthetase; also, the subsequent work of Vogel, Gorini, Maas, and others (cited in ref. 15) showed that repression is not due, as we had thought, to an anti-induction effect. I had always hoped that the regulation of "constitutive" and inducible systems would be explained one day by a similar mechanism. Why not suppose, then, since the existence of repressible systems and their extreme generality were now proven, that induction could be effected by an anti-repressor rather than by repression by an anti-inducer? This is precisely the thesis that Leo Szilard, while passing through Paris, happened to propose to us during a seminar. We had only recently obtained the first results of the injection experiment, and we were still not sure about its interpretation. I saw that our preliminary observations confirmed Szilard's penetrating intuition, and when he had finished his presentation, my doubts about the "theory of double bluff" had been removed and my faith established - once again a long time before I would be able to achieve certainty.

Some of the more important developments of this study, such as the discovery of operator mutants and of the operon, considered as a single coordinated expression of the genetic material, and the bases and demonstration of the messenger theory, have been presented by François Jacob in his lecture[27], and I will not pause over these, in order to return to that constituent whose existence and role had so long escaped me, the repressor. To tell the truth, I find some excuses for myselfeven now. It was not easy to get away completely from the quite natural idea that a structural relation, inherent in the mechanism of the phenomenon of induction, must exist between the inducer of an enzyme and the enzyme itself. And I must admit that, up until 1957, I tried to "rescue" this hypothesis, even at the price of reducing almost to nothing the "didactic" role (as Lederberg would say) of the inducer.

From now on it was necessary to reject it completely. An experiment carried out in collaboration with David Perrin and François Jacob proved, moreover, that the mechanism of induction functioned perfectly in certain mutants, producing a modified galactosidase totally lacking in affinity for galactoside[29].

What now had to be analyzed and understood were the interactions of the repressor with the inducer on the one hand, with the operator on the other. Otto Warburg said once, about cytochrome oxidase, that this protein - or presumed protein - was as inaccessible as the matter of the stars. What is to be said, then, of the repressor, which is known only by the results of its interactions? In this respect we are in a position somewhat similar to that of the police inspector who, finding a corpse with a dagger in its back, deduces that somewhere there is an assassin; but as for knowing who the assassin is, what his name is, whether he is tall or short, dark or fair, that is another matter. The police in this case, it seems, sometimes getresults by sketching a composite portrait of the culprit from several clues. This is what I am going to try to do now with regard to the repressor.

First, it is necessary to assign to the assassin - I mean the repressor - two properties: the ability to recognize the inducer and the ability to recognize the operator. These recognitions are necessarily steric functions and are thus susceptible to being modified or abolished by mutation. Loss of the ability to recognize the operator would result in total derepression of the system. Every mutation that causes a shift in the structure of the repressor or the abolition of its synthesis must therefore appear "constitutive", and this is without doubt the reason for the relatively high frequency of this type of mutation.

However, if the composite portrait is correct, it can be seen that certain

mutations might abolish the repressor's ability to recognize the inducer but leave unaffected its ability to recognize the operator. Such mutations should exhibit a very special phenotype. They would be noninducible (that is, lactose-negative), and in diploids they would be dominant in *cis* as well as in *trans.* Clyde Willson, David Perrin, Melvin Cohn, and I[30] were able to isolate two mutants that possessed precisely these properties, and Suzanne Bourgeois (ref. 31) has recently isolated a score ofothers.

In tracing this first sketch of the composite portrait, I implicitly supposed that there was only one assassin; that is, the characteristics of the system were explained by the action of a single molecular species, the repressor, produced from gene i. This hypothesis is not necessary *a priori.* It could be supposed, for example, that the recognition of the inducer is due to another constituent distinct from that which recognizes the operator. Then we would have to assume that these two constituents could recognize each other. Today this latter hypothesis seems to be practically ruled out by the experiments of Bourgeois, Cohn, and Orgel[31], which show, among other important results, that the mutation of type i^- (unable to recognize the operator) and the mutations of the type i^s (unable to recognize the inducer) occur in the same cistron and, from all appearances, involve the same molecule, a unique product of the regulator gene *i.*

An essential question is the chemical nature of the repressor. Inasmuch as it seems to act directly at the level of the DNA, it seemed logical to assume that it could be a polyribonucleotide whose association with a DNA sequence would take place by means of specific pairing. Although such an assumption could explain the recognition of the operator, it could not explain the recognition of the inducer, because probably only proteins are able to form a stereospecific complex with a small molecule. This indicates that the repressor, that is, the active product of the gene *i,* must be a protein. This theory, based until now on purely logical considerations, has just received indirect but decisive confirmation.

It should be remembered that, thanks to the work of Benzer[32], Brenner[33], and Garen[34], a quite remarkable type of mutation has been recognized, called "nonsense" mutation. This mutation, as is well known, interrupts the reading of the messenger in the polypeptide chain. But on the other hand, certain "suppressors", today well identified, are able to restore the reading of the trip - lets (UAG and UAA) corresponding to the nonsense mutations. The fact that a given mutation may be restored by one of the carefully catalogued suppressors provides proofthat the phenotype of the corresponding mutant is due

to the interruption of the synthesis of a protein. Using this principle, Bourgeois, Cohn, and Orgel[31] showed that certain constitutive mutants of the gene *i* are nonsense mutants and that, consequently, the active product of this gene is a protein.

This result, which illustrates the surprising analytical ability of modern biochemical genetics, is of utmost importance. It must be emphasized that, with respect to the suppression of a constitutive mutant *(i),* it shows that the recognition of the operator (as well as recognition of the inducer) is linked to the structure of the protein produced by the gene *i.*

The problem of the molecular mechanism that permits this protein to play the role of relay between the inducer and the operator still remains. Until now this problem has been inaccessible to direct experimentation, in that the repressor itself remains to be isolated and studied *in vitro.* However, in conclusion, I would like to explain why and how this inaccessibility was itself the source of new preoccupations that we hope will be fruitful.

First of all, is should be recalled that we had tried repeatedly, even before the existence of the repressor was demonstrated, to learn something of the mode of action of the inducer by following its tracks *in vivo* with radioactive markers. One after the other, Georges Cohen, François Gros, and Agnes Ullmann engaged in this approach, using different fractionation techniques. Some of these experiments led to some unexpected and important discoveries, such as that of galactoside permease and galactoside transacetylase. But concerning the way in which galactosides act as inducers, the results were completely negative. Nothing whatever indicated that the inductive interaction is accompanied by a chemical change, however transient, or by any kind of covalent reaction in the inducer itself The kinetics of induction, elaborated on in the elegant work of Kepes[35,36]; also revealed that the inductive interaction is extremely rapid and completely reversible (Fig.5).

This is quite a remarkable phenomenon, if one thinks of it, since this noncovalent, reversible stereospecific interaction - an interaction that in all probability involves only a few molecules and can involve only a very small amount of energy - triggers the complex transcription mechanism of the operon, the reading of the message, and the synthesis of three proteins, leading to the formation of several thousand peptide links. During this entire process, the inducer acts, it seems, exclusively as a chemical signal, recognized by the repressor, but without directly participating in any of the reactions which it initiates.

One would be inclined to consider such an interpretation of the inductive

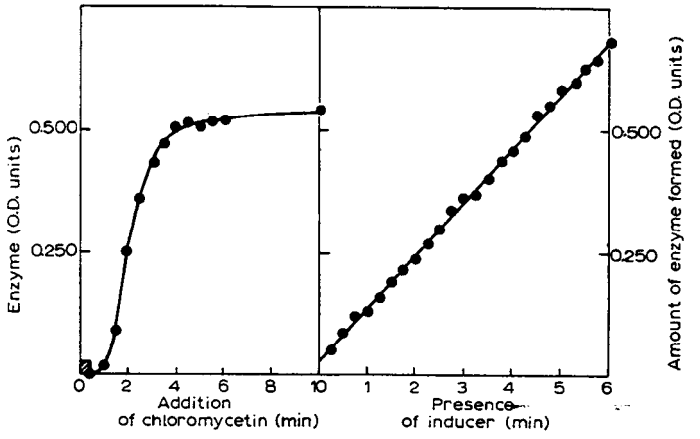

Fig. 5. Kinetics of the synthesis of galactosidase after a short period of induction. Left: Inducer added at time zero. Inducer eliminated after a time corresponding to the width of the cross-hatched rectangle. On the ordinates: accumulation of the enzyme. Right: Total amount of enzyme formed (asymptote of the curve at the left) as a function of the duration of the presence of the inducer. The linear relation obtained indicates that the inductive interaction is practically immediate and reversible[35].

interaction as highly unlikely if one did not know today of numerous examples in which similar mechanisms participate in the regulation of the activity as well as the synthesis of certain enzymes. It was as a possible model of inductive interactions that Jacob, Changeux, and I first became interested in regulatory enzymes[37]. The first example of such an enzyme was undoubtedly phosphorylase *b* from rabbit muscle; as Coris[38] and his group[39] showed, this enzyme is activated specifically by adenosine 5'-phosphate, although the nucleotide does not participate in the reaction in any way. We are indebted to Novick and Szilard[40], to Pardee[41], and to Umbarger[42] for-their discovery of feedback inhibition, which regulates the metabolism of biosynthesis - their discovery led to a renewal of studies and demonstrated the extreme importance of these phenomena.

In a review that we devoted to these phenomena[43], a systematic comparison and analysis of the properties of some of the regulatory enzymes led us to conclude that, in most if not all cases, the observed effects were due to *indirect* interactions between distinct stereospecific receptors on the surface of the protein molecule, these interactions being in all likelihood transmitted by means of conformational modifications induced or stabilized at the time of the formation of a complex between the enzyme and the specific agent - hence the name "allosteric effects", by which we proposed to distinguish this partic-

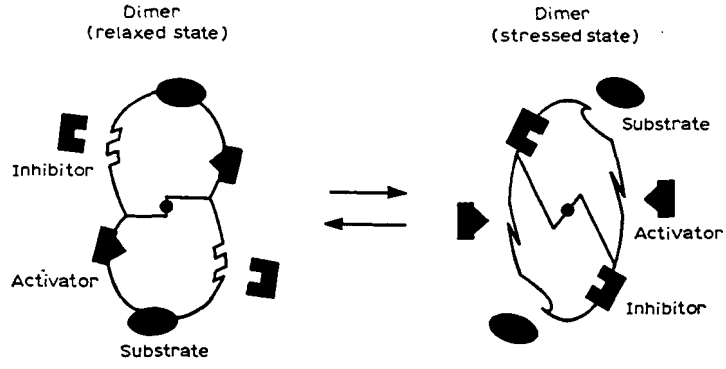

Fig. 6. Model of allosteric transition produced in a symmetrical dimer. In one of the two conformations, the protein can attach itself to the substrate as well as to the activating bond. In the other conformation, it can attach itself to the inhibiting bond.

ular class of interactions, and the term "allosteric transitions", used to designate the modification undergone by the protein (Fig. 6).

By virtue of being indirect, the allosteric interactions do not depend on the structure or the particular chemical reactivity of the ligands themselves, but entirely on the structure of the protein, which acts as a relay. This is what confers upon these effects their profound significance. The metabolism, growth, and division of a cell require, obviously, not only the operation of the principal metabolic pathways-those through which pass the necessary energy and chemical materials - but also that the activity of the various metabolic pathways be closely and precisely coordinated by a network of appropriate specific interactions. The creatin and development of such networks during the course of evolution obviously would have been impossible if only direct interactions at the surface of the protein had been used; such interactions would have been severely limited by chemical structure, the reactivity or lack of reactivity of metabolites among which the existence of an interaction could have been physiologically beneficial. The "invention" of indirect allosteric interactions, depending exclusively on the structure of the protein itself, that is on the genetic code, would have freed molecular evolution from this limitation[43].

The disadvantage of this concept is precisely that its ability to explain is so great that it excludes nothing, or nearly nothing; there is no physiological phenomenon so complex and mysterious that it cannot be disposed of, at least on paper, by means of a few allosteric transitions. I was very much in agreement with my friend Boris Magasanik, who remarked to me several years ago that this theory was the most decadent in biology.

It was all the more decadent because there was no *a priori* reason to suppose that allosteric transitions for different proteins need be of the same nature and obey the same rules. One might think that each allosteric system constituted a specific and unique solution to a given problem of regulation. However, as experimental data accumulated on various allosteric enzymes, surprising anal - ogies were found among systems that had apparently nothing in common. In this respect, the comparison of independent observations by Gerhart and Pardee[44] on aspartate transcarbamylase and by Changeux[45] on threonine deaminase of *Escherichia coli* was especially impressive. By their very complexity, the interactions in these two systems presented unusual kinetic characteristics, almost paradoxical and yet quite analogous. Therefore it could not be doubted that the same basic solution to the problem of allosteric interactions had been found during evolution in both cases; it remained only for the researcher to try to discover it in his turn.

Among the properties common to these two systems, as well as to the great majority of known allosteric enzymes, the most significant seemed to us to be the fact that their saturation functions are not linear (as is the case for "classic" enzymes) but multimolecular. An example of such a pattern of saturation has been known for a long time: it is that of hemoglobin by oxygen (Fig. 7). Jeffries Wyman had noted several years earlier[46] that the symmetry of the saturation curves of hemoglobin by oxygen seemed to suggest the existence of a

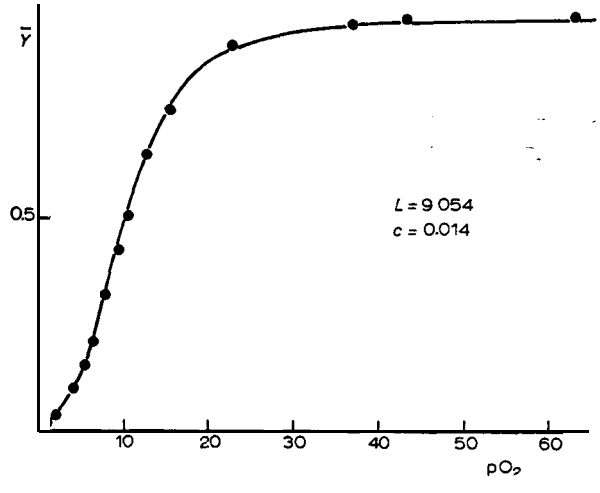

Fig. 7. Saturation of hemoglobin with oxygen. Abscissa: partial pressure of O_2. Ordinate: saturated fraction. The points correspond to experimental points[51]. The interpolation curve was calculated from a theoretical model essentially similar to that of Fig. 6.

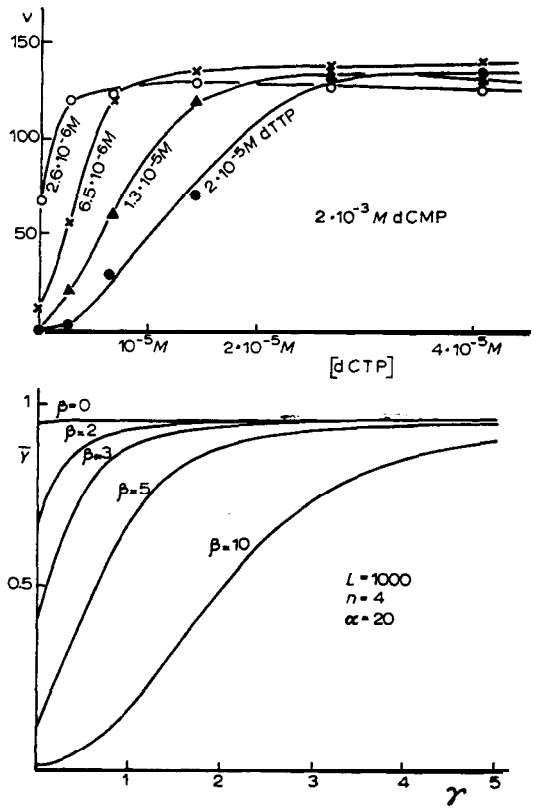

Fig. 8. Activity of deoxycytidine deaminase as a function of the concentration of the substrate (dCMP), of the activator (dCTP), and of the inhibitor (dTTP). (Top) Experimental results (from Scarano; see ref. *48*). (Bottom) Theoretical curve calculated for a similar case according to the model of Monad, Wyman and Changeuxe[48].

structural symmetry within the protein molecule itself; this idea was brilliantly confirmed by the work of Perutz[47].

These indications encouraged us- Wyman, Changeux, and myself-to look for a physical interpretation of the allosteric interactions in terms of molecular structure. This exploration led us to study the properties of a model defined in the main by the following postulates:

(1) An allosteric protein is made up of several identical subunits (protomers).

(2) The protomers are arranged in such a way that none can be distinguished from the others; this implies that there are one or more axes of molecular symmetry.

(3) Two (or more) conformational states are accessible to this protein.

(4) These conformational transitions tend to preserve the molecular symmetry, or, more generally, the equivalence of the protomers[48].

We were pleasantly surprised to find that this very simple model made it possible to explain, classify, and predict most of the kinetic properties, sometimes very complex in appearance, of many allosteric systems (Figs. 7 and 8). Obviously, this model represents only a first approximation in the description of real systems. It is not likely, moreover, that it represents the only solution to the problem of regulative interactions found during evolution; certain systems seem to function according to quite different principles[49], which will also need to be clarified.

However, the ambition of molecular biology is to interpret the essential properties of organisms in terms of molecular structures. This objective has already been achieved for DNA, and it is in sight for RNA, but it still seems very remote for the proteins. The model that we have studied is interesting primarily because it proposes a functional correlation between certain elements of the molecular structure of proteins and certain of their physiologic properties, specifically those that are significant at the level of integration, of dynamic organization, of metabolism. If the proposed correlation is experimentally verified, I would see an additional reason for having confidence in the development of our discipline which, transcending its original domain, the chemistry of heredity, today is oriented toward the analysis of the more complex biological phenomena: the development of higher organisms and the operation of their networks of functional coordinations.

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