

# General Conclusions: Teleonomic Mechanisms in Cellular Metabolism, Growth, and Differentiation

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## I. INTRODUCTION

Before attempting to draw the conclusions, or some of the conclusions, which emerge from the discussions of the past eight days, we would like to express the unanimous feeling of the participants that the choice of the subject and the timing of this conference were excellent, as shown by the exceptional and sustained interest of the sessions. For this we are deeply indebted to our host Dr. Chovnick, Director of the Long Island Biological Laboratory, and to Dr. Umbarger who had a major share in the planning of the conference.

We shall not attempt here to summarize the proceedings of a meeting where such an abundance of observations, pertaining to a wide variety of systems, were presented. We would rather try to reconsider the problem of cellular regulation as a whole, in perspective so to say, as it appears to us as a result of this confrontation.

One conclusion which was repeatedly emphasized is the wide-spread occurrence and the extreme importance of regulatory mechanisms in cellular physiology. Since this aspect has been treated, with characteristic elegance and insight by Dr. B. Davis, in his introductory paper, we shall not dwell on it here. Let us however recall, for instance, the systems described by Dr. Kornberg (see this Symposium, page 257) which illustrate the fact that essential enzymes of intermediary metabolism, such as the condensing enzyme (a typical "amphibolic" enzyme according to the useful terminology proposed by Davis), are submitted to wide regulatory variations, depending on the substrates present in the medium. The idea, often expressed in the past, that adaptive effects are limited to "unessential" enzymes is thus evidently incorrect. Let us also recall that the genetic breakdown of a regulatory mechanism has repeatedly been found (cf. the cases of  $\beta$ -galactosidase, alkaline phosphatase, aspartate and ornithine transcarbamylase) to lead to enormous overproduction of the enzyme concerned; it is evident that no cell could survive the breakdown of more than two or three, at most, such systems. Finally, let us also point to the wide variations observed, in relation to different diets, in the level of liver enzymes, and to the significant observation that, in certain hepatic tumors, the same enzymes appear to obey al-

together different rules of conduct (see Van Potter, this Symposium, page 355).

In the present discussion, we wish to center attention on the mechanisms, rather than on the physiological significance, of the different regulatory effects. It is clear that great progress has been accomplished in this respect, allowing us now clearly to distinguish between different types of mechanisms, and also to recognize that certain systems which appeared entirely different from one another a few years ago, are in fact submitted to similar, if not identical, controls. This is particularly striking in the case of inducible and repressible enzyme systems and of lysogenic systems, all three of which would seem to obey fundamentally similar controlling elements, merely organized into different circuits.

The major part of this paper will then be devoted to the discussion of mechanisms. However, the analysis of these mechanisms has been, so far, largely restricted to microbiological objects. A constantly recurring question is: to what extent are the mechanisms found to operate in bacteria also present in tissues of higher organisms; what functions may such mechanisms perform in this different context; and may the new concepts and experimental approaches derived from the study of microorganisms be transferred to the analysis and interpretation of the far more complex controls involved in the functioning and differentiation of tissue cells? We shall consider this question in the last section of this paper.

## II. REGULATORY MECHANISMS

### A. POSSIBLE, PLAUSIBLE, AND ACTUAL CELLULAR CONTROL MECHANISMS

To begin with, we might try to classify and define *a priori* the main types of cellular regulatory mechanisms, including any likely or plausible mechanism which may or may not have been actually observed, or discussed during the present conference.

#### 1. Mass action

Since many, if not most, metabolic reactions are largely reversible, mass action might have a significant share in regulation. However most pathways involve one or several irreversible steps which could not be

controlled by mass action. Moreover, it is a general observation that the intracellular concentration of most intermediary metabolites in the cell is vanishingly small, indicating that mass action only plays a limited role, and also suggesting that other mechanisms must intervene in metabolic regulation. Mass action effects were, in fact, not discussed during this conference.

## 2. Enzyme activity

By virtue of the buffering effect implied by Henri-Michaelis kinetics, an enzyme constitutes, by itself, a controlling element. The rate of the reaction which it catalyzes depends upon its characteristic kinetic constants, in particular on its relative affinity for substrate and product. It is worth noting that these constants are related to the equilibrium constant, i.e., to the free energy change of the reaction itself, by the Haldane equation, thus reintroducing mass action as one of the controlling factors in any enzyme-catalyzed reaction. The relative values of the forward and backward reaction constants in the Haldane equation may be supposed to present, in some systems at least, a physiological, controlling significance. For instance, the fact that alkaline phosphatase, which catalyzes a virtually irreversible reaction, has a very high affinity for orthophosphate may result in control of this reaction by the product, in spite of irreversibility. The "teleonomic" significance of this correlation, where it obtains, is emphasized by the fact that in other irreversible systems, the enzyme shows very low affinity for the products. This is the case, for instance, for the  $\beta$ -galactosidase reaction. Thus, intracellular phosphate esters may be protected by intracellular orthophosphate, while galactosides would not be so protected by galactose. The products of an enzyme necessarily are *analogues* of the substrate, and competitive inhibition is expected in any case: whether it is physiologically significant or not depends upon the specific construction of the enzyme site.

Competitive inhibition of enzymes by organic substances other than steric analogues of the substrate (including product) is not observed, in general. But the specific construction of enzyme sites offers yet other regulatory possibilities, as revealed by the discovery of the "feedback" or "endproduct" inhibition effect. As we have seen, this type of effect actually turns out to be extremely wide-spread and physiologically highly significant. We shall discuss it at some length.

## 3. Enzyme activation and "molecular conversion"

The well known conversion of zymogens into active proteases evidently plays an important regulatory and protective role. On this basis, one might expect various types of alteration of molecular structure ("molecular conversion") to occur in the regulation of activity of intracellular enzymes. Actually, relatively few observations of such effects have been reported. However, the

mechanisms described by Tompkins and by Rall and Sutherland may be considered as "molecular conversions" and this may also be true of the effects reported by Hagerman. We shall discuss the possible implications of these mechanisms in a later section.

## 4. Specific control of enzyme synthesis

Since it is well known that cells of different tissues within the same organism do not exhibit the same enzyme (or protein) patterns, while all these cells presumably contain the same genome; and since the same may be said of bacteria from a single clone grown in different media, it is evident that specific mechanisms exist, which control the expression of genetic potentialities with respect to specific protein synthesis. In bacteria, adaptive enzyme systems have been the subject of much work, and we shall discuss these systems at some length. The occurrence of similar mechanisms in differentiated organisms is highly probable, although, as the discussions here have shown, not conclusively demonstrated in any single case. It would appear that some of the "adaptive" effects observed in tissue cells are due to enzyme stabilization rather than to control of enzyme synthesis. This will be discussed in the last section of this paper.

From this brief review and classification of the main plausible and/or actually observed mechanisms of cellular control, it is apparent that *all* these mechanisms—except mass action—are directly related to the specific molecular structure of the enzymes, or other proteins, concerned. The fundamental problem of specific determinism in protein synthesis is, therefore, coextensive to our field of investigation. This would be the justification, if any were needed, for the fact that a major part of this conference was devoted to this problem. We shall discuss it in connection with enzymatic adaptation since, as we have seen, induction and repression are directly related to the mechanisms of information transfer from genes to proteins.

## B. THE NOVICK-SZILARD-UMBARGER EFFECT: *Endproduct or "Allosteric" Inhibition*

In 1954, Novick and Szilard discovered that the synthesis of a tryptophan precursor (later identified as indol-3-glycerol-phosphate) in *E. coli* was inhibited by tryptophan. They formulated the hypothesis that tryptophan specifically inhibited the activity of an *early* enzyme in tryptophan biosynthesis and that this effect had regulatory significance. Observations of the Carnegie group on isotopic competition (Roberts *et al.*, 1955) between endogeneous and exogeneous metabolites suggested the occurrence of similar effects in the synthesis of several amino acids. The work of Umbarger (see this Symposium, page 301), directly at the enzyme level, indeed demonstrated that, in many pathways, an early enzyme is so constructed as

to be strongly and specifically inhibited by the metabolic endproduct of the pathway.

As the reports here have shown, endproduct inhibition is extremely widespread in bacteria, insuring immediate and sensitive control of the rate of metabolite biosynthesis in most, if not all, pathways. From the point of view of mechanisms, the most remarkable feature of the Novick-Szilard-Umbarger effect is that the inhibitor is *not a steric analogue of the substrate*. We propose therefore to designate this mechanism as "allosteric inhibition." Since it is well known that competitive behavior toward an enzyme is, as a rule, restricted to steric analogues, it might be argued that an enzyme's concept of steric analogy need not be the same as ours, and that proteins may see analogies where we cannot discern any. That this interpretation is inadequate is proved by many observations which were reported here. Umbarger and others have shown that in general, only *one* enzyme, the first one in the specific pathway concerned, is highly sensitive to inhibition by the endproduct. If steric analogy were involved, the different enzymes of the pathway would then be considered to hold private and dissenting opinions about stereochemistry. And the same would have to be said of two different enzymes catalyzing an *identical* reaction in the *same* organism, as in the remarkable case of  $\beta$ -aspartokinase, reported by Cohen and Stadtman (see this Symposium, page 319).

Such observations leave no doubt that the construction of the binding site of enzymes subject to allosteric inhibition is exceptional and highly specialized. The findings of Changeux (see this Symposium, page 313) actually show that the groups involved in the binding of inhibitor, in the case of threonine-deaminase, may be inactivated without parallel inactivation of the enzyme. They show, moreover, that the abnormal reaction kinetics of this enzyme (already noted by Umbarger) are directly related to its competence as a regulatory enzyme, and may be experimentally normalized by inactivation of the inhibitor binding groups. This leads to the conclusion that two distinct, albeit interacting, binding sites exist on native threonine deaminase. Competitive inhibition in this system, therefore, is not due to *mutually exclusive* binding of inhibitor and substrate, as in the classical case of steric analogues.

Closely similar observations have been made independently and simultaneously by Pardee (private communication) on another enzyme sensitive to endproduct (aspartate-carbamyl-transferase). This situation may therefore be a general one for enzymes subject to allosteric inhibition and these findings raise several interesting new problems of enzyme chemistry. Studies of the structure of the two sites and of their interaction, using analogues of the substrate and inhibitor, might conceivably lead to interpretations in terms of the "induced-fit" theory of Koshland (1959).

In any case, one may predict that "allosteric enzymes" will become a favorite object of research, in the hands of students of the mechanisms of enzyme action.

Since the allosteric effect is not *inherently* related to any particular structural feature common to substrate and inhibitor, the enzymes subject to this effect must be considered as pure products of selection for efficient regulatory devices. This raises a question concerning the genetic determinism of allosteric enzymes. If indeed these enzymes generally possess two different binding groups, they might be supposed to represent the association, favored by selection, of two originally independent enzyme-proteins. If such were the case, one might expect the structural gene corresponding to such an enzyme to be, as a rule, composed of two cistrons, governing respectively the structure of each of the two components of the molecule. In vitro dissociation and reassociation of the two components might also be observed, and would help greatly in the analysis of the effect itself.

A particularly interesting possibility is suggested by this discussion. Namely that, since again there is no obligatory correlation between specific substrates and inhibitors of allosteric enzymes, the effect *need not be restricted to "endproduct" inhibition*. (This in fact is the main reason for avoiding the term "endproduct inhibition" in a general discussion of this mechanism. We feel that endproduct inhibition may turn out to constitute only *one class* of allosteric effects.) It is conceivable that in some situations a cell might find a regulatory advantage in being able to control the rate of reaction along a given pathway through the level of a metabolite synthesized in another pathway. Wherever favorable, such "cross inhibition" might have become established through selection. In other words, *any* physiologically useful regulatory connection, between any two or more pathways, might become established by adequate selective construction of the interacting sites on an allosteric enzyme. This, we feel, may be a very important point, to which we shall return later.

Another aspect should be mentioned. As is well known, the principle of steric analogy has been widely used in attempts to rationalize the design of synthetic drugs, particularly in the case of antibacterial and antitumoral agents. The results have been rewarding, although not as much, perhaps, as one might have anticipated. Yet the principle is evidently valid. But it may prove even more rewarding to look for analogues of the natural controlling agent, rather than for analogues of the substrate of the reaction which one proposes to hit. An example of such an analogue is furnished by 5-methyl-tryptophan, which does not compete with tryptophan for incorporation into protein, while it does efficiently block tryptophan synthesis by allosteric inhibition (and also by repression) (Trudinger and Cohen, 1956).

Similar considerations evidently apply to the analysis of the mode of action of drugs and antibiotics.

### C. MOLECULAR CONVERSION

As we already noted, the well-known example of the zymogens seemed to suggest that alterations, reversible or not, of the molecular structure of certain enzymes might represent an important type of regulatory mechanism. Surprisingly enough, very few examples of such mechanisms have been discovered. It would be unwise to conclude that "molecular conversions" are not a significant type of mechanisms, especially in view of some of the observations reported here. Tomkin's work on the glutamic-alanine dehydrogenase conversion (see this Symposium, page 331) does more than reveal a possible mechanism of steroid hormone action. His observations show that the same protein may acquire different specific activities, depending upon a reversible alteration of molecular structure. This discovery would seem for the first time to justify the idea, often expressed in the past, that an enzyme might possess, *in vivo*, several different activities (alternative or not) which might be difficult to recognize *in vitro*. In Tomkin's case, the conversion involves interaction of the protein with itself. In other cases, it might conceivably depend upon interaction of two different proteins, and might remain undetected for this reason. Such possibilities are also suggested by the work of Yanofsky on the two components of tryptophan synthetase. Whether or not the glutamic-alanine dehydrogenase conversion affords a physiologically valid interpretation of steroid action, it does propose a model of a possibly important type of regulatory mechanism.

To a certain extent, the phosphorylase "conversion" discussed here by Rall and Sutherland (see this Symposium, page 347) pertains to the same general type of mechanism, since the activity of phosphorylase eventually depends upon its interaction with two other specific proteins, which phosphorylate and dephosphorylate respectively the metabolic enzyme. (In passing, it may be of interest to note that certain types of suppressor mutations could be due to interactions of this type.) It will be interesting to see whether the transhydrogenase activation, described by Hagerman, also belongs to the class of molecular conversions. In microorganisms, the formation (induced by aerobic conditions) of L-lactic from D-lactic dehydrogenase has been reported by Labeyrie, Slonimsky and Naslin (1959). Whether or not this is pure molecular conversion, or involves *de novo* synthesis of part of the enzyme molecule is not established as yet.

We would venture to predict that in the next few years several new examples of molecular conversions will be discovered.

Little has been said during this conference of the

mechanisms which control cell division. It should be noted that these mechanisms presumably involve, or govern, certain types of "molecular conversions." This is most clearly indicated by the work of Mazia (1959) following the pioneer investigations of Rapkine (1931). Lwoff and Lwoff (1961) have stressed the fact that in the cycle of the polio virus, cyclic dissociation and association of the coat protein occurs, and they have suggested that similar events, affecting certain proteins, may play an important role in cell division. Systematic inquiries based upon this suggestion would certainly be justified.

### D. SPECIFIC CONTROL OF PROTEIN SYNTHESIS

#### 1. *The determinism of protein structure*

The discussions at this conference have shown, once more, that the one gene-one enzyme hypothesis is now considered as established beyond reasonable doubt. The early difficulties of the theory were evidently due to insufficient biochemical analysis of the apparent exceptions. In the case of several enzyme-proteins, known to be made up of two or more polypeptide chains, it is now apparent that the structure of each polypeptide chain is governed by an independent gene or cistron. This constitutes a remarkable confirmation of the theory and an important step forward in understanding the mechanisms which govern protein structure. The work of Yanofsky (see this Symposium, page 11) on tryptophan-synthetase has been particularly illuminating in this respect.

Even when the one gene-one enzyme theory is redefined and qualified as the one cistron-one polypeptide chain theory, some complications remain, the interpretations of which are still not elucidated. We refer to intracistronic complementation and to the occurrence of suppressor mutations.

Although the first problem, intracistronic complementation, was not discussed during this conference, it should be briefly mentioned here. It is now generally believed to be often associated with a polymeric state of the normal enzyme protein. Observations made with a number of complementary mutants of glutamic dehydrogenase (Fincham, 1959) and  $\beta$ -galactosidase (Pasteur group) are in keeping with this assumption. The active enzyme, in both cases, is known to be a polymer, while certain mutations, in the case of  $\beta$ -galactosidase, result in the formation of an inactive monomer (Perrin, 1961). Studies of *in vitro* complementation may be expected to throw much light on the building of tertiary and quaternary structures of proteins. In any case, intracistronic complementation does not seem to offer a serious challenge to the concept that the gene or cistron acts as a *unit* in determining polypeptide structure.

The difficulty of interpreting suppressor mutations appears to be much greater. It has generally been as-

sumed that suppressor mutations acted in some way at the tertiary level of protein synthesis, in contrast to true structural mutations assumed to operate at the primary level. The observations reported by Yanofsky indicate that certain suppressor mutations may actually restore the wild-type peptide structure in a fraction of the molecules. The working hypothesis proposed by Yanofsky following earlier suggestions of Benzer (namely that these suppressor mutations modify the specificity of an amino acid-activating-enzyme in such a way that compensatory errors would occur with a certain frequency in the choice of the corresponding amino acid) appears particularly interesting since it involves precise predictions. One of these predictions of course is that in such mutants the properties of one of the 20-odd amino acid activating enzymes should be detectably modified. If so, proof would be virtually obtained that the corresponding sRNA fraction does play the role of an adaptor as assumed by Crick (1958) and others, and a new method of determining amino acid substitutions resulting from structural mutations might become available. Another prediction is that the same suppressor mutation might be found to correct in part the effects of two primary mutations affecting two different enzymes. And lastly one would not expect such suppressor mutations to occur at more than about 20 loci. Thus, confirmation of Yanofsky's hypothesis will be awaited with particular interest.

The two fundamental problems with which we are now faced are the nature of the code and the mechanisms of information transfer from DNA to enzyme-synthesizing centers.

A few years ago, following the beautiful work of Benzer (1957) which demonstrated the linear structure of the genetic material at the ultrafine level and the work of Ingram (1957) on sickle-cell hemoglobin, it seemed that the basic assumption of all coding hypotheses, namely collinearity, would soon be proved. The only proof that has been obtained so far is that optimism is essential to the development of Science; collinearity still remains to be formally demonstrated. However, the reports of Yanofsky, of Streisinger and of Rothman at the conference, and what is known of the work of other laboratories, notably Brenner's, again encourage optimism; one feels confident that the final demonstration will soon be at hand.

The nature of the code itself is another matter. But the new experimental approaches, notably the study of chemical mutagens, are developing so rapidly (cf. Benzer and Freese, 1958; Freese, 1959) that cautious and patient optimism is justified. The study of the effects of reverse mutations occurring at the same site as the primary alteration, may also permit the elimination of certain types of codes. Finally a direct, chemical attack, involving the determination of partial (terminal) sequences in both a protein and the corre-

sponding messenger RNA, may become possible, assuming the mRNA theory to be correct, if and when methods of isolating a specific message will be available.

A new experimental approach to the problem of the universality, or otherwise, of the code has been opened up by the observation of Falkow *et al.* (1961) of genetic transfer between *E. coli* and *Serratia*. Preliminary observations by the Pasteur Institute and M.I.T. groups on  $\beta$ -galactosidase and alkaline phosphatase suggest that the *E. coli* genes are transcribed correctly in *Serratia*. This would seem to indicate that the 20% difference in the G + C/A + T ratio between the two genera is not due to the use of different codes, and would agree with Sueoka's universalist conclusions. Further and more detailed studies of proteins synthesized by such "displaced" genes are evidently required. If the codes in *Serratia* and *Escherichia* and perhaps a few other bacterial genera turn out to be the same, the microbial-chemical-geneticists will be satisfied that it is indeed universal, by virtue of the well-known axiom that anything found to be true of *E. coli* must also be true of Elephants.

However, the remarks of Benzer, and also Yanofsky's interpretation of his suppressor mutations suggest that discrete differences of coding, concerning only one or a few amino acids, might exist between different groups, due to differences in specificity of the activating enzymes. The possibility that the code is universal for certain amino acids, and non-universal for others, seems interesting from an evolutionary point of view.

Assuming the problem of the code to be advancing, albeit slowly, the problem of how the tertiary structures are determined remains very open. But while this question was posed only in general terms until recently, it is now very precisely defined by the beautiful studies of the Cavendish group on the structures of myoglobin and of the  $\alpha$  and  $\beta$  chains of hemoglobin. These studies have revealed that the tertiary structure of all three polypeptide chains are closely similar, while the primary structure of myoglobin differs widely from that of both hemoglobin chains, except however for about twelve residues which appear to occupy identical strategic positions in the three proteins (Perutz, 1961). This is a remarkable confirmation of the idea (Crick, 1958) that the tertiary folding is governed by a certain number of key residues, while being largely independent of the nature of residues in other positions. It remains to be seen whether it will ever be possible to formulate any general "folding rules" which would allow one approximately to deduce the tertiary configuration of a protein from knowledge of its primary structure. Yet, this is the goal that one would wish to reach, since this deduction, which we cannot begin to make, seems to be made unflinchingly by the protein-synthesizing machinery in the cell.

This brings up another issue which must be mentioned at this point, although it was not discussed during the conference, evidently because it is implicitly considered as settled. A few years ago, the question was often debated whether any further (non-genetic) *structural* information needed to be furnished, or might conceivably be used in some cases, at the stage of tertiary folding in protein synthesis. Such a "finishing touch" has been considered as one of the possible mechanisms which might account for the effect of antigen in antibody synthesis (Pauling, 1940) and of inducer in enzymatic adaptation (Monod and Cohn, 1952). In the latter case, no evidence for, and a great deal of evidence against this possibility has accumulated (cf. Monod, 1956, Jacob and Monod, 1961) and proof has been obtained that inducer action is completely unrelated to the structure of the binding site of the induced enzyme (Perrin *et al.*, 1960). In the meantime, speculations on the origin of antibodies reverted from "instructive" to purely "selective" theories (Burnet, 1959; Lederberg, 1959). While this evolution is justified, in the case of antibodies, by general considerations, direct experimental evidence is yet to be found that would allow "selection" of the correct theory.

## 2. The control of gene expression

As we already pointed out, the purely structural (one gene-one enzyme) theory does not consider the problem of gene expression. The discovery of a new class of genetic elements, the regulator genes, which control the *rate* of synthesis of proteins, the *structure* of which is governed by *other* genes, does not contradict the classical concept, but it does greatly widen the scope and interpretative value of genetic theory. In all the adequately studied cases, it is established that the regulator genes act negatively (i.e. by blocking rather than provoking the synthesis of the proteins which they control) through the intermediacy of a cytoplasmic "aporepressor". Although the chemical nature of the aporepressor is still unknown, we feel that the term "regulator gene", as operationally defined, for instance, in the case of the lactose system of *E. coli*, should not be applied indiscriminately to any gene found to influence, in an unknown way, the formation of an enzyme: it is clear that a *structural* gene might exert such an effect by, e.g. controlling an enzyme which synthesizes an inducer of another system (cf. the observations of Horowitz in this Symposium, page 233).

To avoid confusion, the term "regulator" should be applied only to genes identified by *recessive constitutive mutations* affecting a protein structurally controlled by *another gene*.

In any case, the most urgent problem with respect to regulator genes is to identify their active product. Although it is almost certain that this product cannot

be a small molecule, and while it seems likely that it is not a protein, there is no positive evidence to identify it as a nucleic acid. Only when this question is solved shall it be possible to study directly the interaction of inducer or repressor with aporepressor, and to account for the specificity of this interaction.

Concerning this last point, the only statement that can be made at present is a strictly negative one: namely that the specificity of induction or repression is completely independent of the specificity of action of the enzymes involved. Although inducers are in general substrates, or analogues of the substrate, and repressors are products (often distant) of the controlled enzyme, the mechanism of the effect itself imposes no restriction upon the "choice" of the active agent. The specificity therefore must be considered purely as a result of selection, as in the case of allosteric inhibition. This selective freedom may have some important theoretical implications which will be discussed later.

As we have seen (Jacob and Monod, this Symposium, page 193) there are very strong reasons to believe that the site of action of the repressor is genetic; that in fact it is identical with the "operator" locus itself. Besides the arguments derived from the kinetics of enzyme synthesis, to which we shall return, the main reason is the existence, in certain systems, of genetic units of coordinate expression, i.e. of "operons" including several structural genes, controlled by a single operator. So far, operons have been recognized only in bacteria, where genes controlling sequential enzymes are frequently, if not generally, tightly clustered (Demerec and Hartmann, 1959). One may wonder whether the concept of operon also applies to organisms where genetic clustering is not usually observed. The fact that pseudoalleles have been discovered in *Drosophila* and maize, wherever genetic methods attained sufficient resolution, suggests that the clustering of cistrons involved in controlling the same biochemical step may in fact be very widespread. It is tempting to speculate that the loci where pseudoallelism is observed control the synthesis of proteins containing two or more different polypeptide chains and that they involve two or more linked cistrons. Thus the operon, in higher organisms, might often correspond to the "gene" as defined by the one gene-one enzyme concept. Moreover, as we have seen, the results obtained with bacteria also permit one to define the operon in a somewhat different manner, namely as the *unit of transcription*. This definition remains valid and useful independently of the number of cistrons covered by a given operon.

Long before regulator genes and operator were recognized in bacteria, the extensive and penetrating work of McClintock (1956) had revealed the existence, in maize, of two classes of genetic "controlling elements" whose specific mutual relationships are closely comparable with those of the regulator and operator: the

"Activator" of McClintock appears to work as a *transmitter* of signals, presumably cytoplasmic since they act both in *cis* and in *trans*. By contrast the specific *receiver* of these signals only acts in *cis* upon genes directly linked to it. Although, because of the absence of enzymological data in the maize systems, the comparison cannot be brought down to the biochemical level, the parallel is so striking that it may justify the conclusion that the rate of structural gene expression is controlled, in higher organisms as well as in bacteria and bacterial viruses, by closely similar mechanisms, involving regulator genes, aporepressors, operators and operons.

A last point concerning the operator should be made. As we have seen, the operator locus of the Lac operon in *E. coli*, appears to be part of one extremity of the structural gene controlling galactosidase. In the arginine system (see Vogel; Maas; and Gorini; this Symposium) a single regulator appears to control the expression of several unlinked genes (or clusters) governing the different enzymes of the sequence. The operator segment for each of these genes or clusters presumably has the same structure, and if so one would expect the different enzymes of the system to contain the same sequence in one of their terminal peptides. Apart from the interest of providing a possible test for the preceding assumptions, the evolutionary implications of such a situation are evident.

### 3. Messenger RNA

The assumption that regulation, in inducible and repressible systems, operates at the genetic level by blocking or releasing the synthesis of the primary genetic product is intimately related to the problem of "messenger-RNA". On the basis of the kinetics of induction and repression, this assumption necessarily implied that the primary product in question is a short-lived intermediate (Jacob and Monod, 1961) and it led to a systematic search for an intermediate endowed with the proper kinetic properties. As we have seen, this search has been remarkably successful.

All or most of the evidence available at present on the so-called "messenger-RNA" fraction has been discussed in detail during the conference and we need not consider it at any length here. It might be useful however to summarize the main conclusions as follows:

a. A RNA fraction endowed with an exceptionally high rate of turnover exists not only in phage-infected cells (Volkin and Astrachan, 1957) but also in normal cells (Gros *et al.*, see this Symposium, page 111).

b. The base ratios in this fraction, in contrast to all other RNA fractions approximate the characteristic (group specific) base ratios of DNA (Volkin and Astrachan, 1957; Yčas and Vincent, 1960; Hayes, Hayes and Gros, 1961).

c. "mRNA" appears to form hybrids with homologous but not with heterologous DNA, indicating that

the sequences in "mRNA" complement the sequences in DNA (Spiegelman, see this Symposium, page 75).

d. An enzyme system able to synthesize RNA polynucleotides using DNA as primer and reproducing the DNA base ratios in its product exists in *E. coli* from which it has been isolated and purified (Hurwitz *et al.*, see this Symposium, page 91).

e. *Escherichia coli* ribosomes appear to be able to synthesize either bacterial protein or viral protein depending on whether the "mRNA" with which they are associated is viral or bacterial; in other words, ribosomes appear to be non-specific with respect to the type of protein which they synthesize. (Brenner *et al.*, see this Symposium, page 101).

f. In reconstructed subcellular systems, the presence of DNA appears essential both for the incorporation of amino acid into protein, and for the synthesis of RNA, presumably mRNA, as shown in particular by Tissières' recent results; in the absence of DNA, partially isolated mRNA stimulates incorporation.

The very significant recent findings of Wood, Chamberlain and Berg (1961, in preparation) should be recalled here although they were not discussed at the conference. Using reconstructed systems containing washed ribosomes, they found that amino acid incorporation into protein was almost completely dependent upon the addition of purified polymerase, DNA, and triphosphonucleotides, the absence of any one of these additions resulting in 90 to 95% inhibition of incorporation.

The sum of these observations is impressive and seems to justify the optimistic feeling shared by most of us that the primary product of the genes, the intermediate responsible for the transfer of structural information to protein-forming centers, has been identified, as well as the enzyme system which synthesizes this product by transcribing DNA into RNA. However it must be pointed out that formal proof of the structure-determining function of "mRNA" will be obtained only when the synthesis of a specific protein, known to be controlled by an identified structural gene, is shown to take place in a reconstructed system containing messenger-RNA from genetically competent cells, while all other fractions were prepared from cells known to lack this particular structural gene.

It should also be emphasized that, while the existence of a fraction possessing the properties of "mRNA" was predicted largely on the basis of the assumption that repressive regulation operates at the genetic level, it remains to be proved, also by direct experiments, that inducers and repressors do control the synthesis of the specific messengers corresponding to the proteins which they are known to induce or repress *in vivo*.

Many other problems are raised by the recent findings on messenger-RNA. One of them is the stoichi-

ometry of the intermediate. The possibility that the stoichiometry is one to one (that is to say that one molecule of messenger is destroyed for each molecule of protein synthesized) is interesting, but it seems to meet with serious difficulties. The possibility that the messenger may be endowed with different stability in different species or groups is at least equally likely, and it may eventually be found to account for the conflicting reports in the literature concerning the effects of enucleation on protein synthesis.

A question which was in the minds of many participants of the meeting was what the role of ribosomes and ribosomal RNA in protein synthesis might be, if indeed all of the specific structural information is provided by mRNA. Among various speculations, for which there is at present no basis and little immediate hope of devising experimental tests, one may mention the possibility that ribosomal RNA can form base pairing bonds with mRNA and thereby stretch it into the correct position for protein synthesis. In addition, the configuration in space of the ribosome-mRNA complex might restrict the freedom of folding of the polypeptide chain and thereby provide certain folding rules.

#### E. THE GLUCOSE EFFECT

One of the oldest known regulatory effects in enzyme synthesis is generally known today as the "glucose effect" although it is recognized that almost any carbon source may inhibit the synthesis of a wide variety of enzymes, the magnitude of the inhibition depending mostly on the rate of metabolism of the compound. The widespread occurrence and the physiological importance of this effect were illustrated in particular by Magasanik's report (see this Symposium, page 249). Concerning mechanisms however, few conclusions can be drawn at present. The most urgent question in this respect is whether the inhibition by glucose, or other carbon sources, of synthesis of an inducible enzyme is related or not to the mechanism of induction itself. The data summarized by Brown would seem to indicate that, in contrast with previous views, the glucose effect is largely independent of the specific aporepressor-inducer interaction. Brown's findings (Brown and Monod, 1961) would be consistent with a model involving the synthesis, in the presence of glucose, of a more or less non-specific inhibitory compound, indifferent to the presence or absence of the specific aporepressor as well as of the inducer.

The findings of Magasanik and of Neidhardt (see this Symposium, page 249 and 63) on the other hand indicate that the inhibitory agent ultimately responsible for the glucose effect must have some degree of specificity. On the basis of the knowledge acquired concerning the mechanism of specific induction and repression, it would seem that the following questions,

concerning the nature of the glucose effect, should be asked and could receive an experimental answer:

a. Is the inhibitory agent specific for certain groups of enzymes? If it is, one would expect to find mutants which have lost the capacity to synthesize this compound and therefore would have lost the glucose effect for certain types of enzymes while retaining it for others.

b. Does the inhibitory agent act at the same level as the specific aporepressor? If so, certain mutations in the operator region might modify quantitatively the glucose effect towards enzymes belonging to the corresponding operon.

c. If the glucose effect does *not* work at the operator level, but rather at the cytoplasmic level (as suggested by some findings of Halvorson (discussion at this Symposium, see page 231), the quantitative regulatory coordination within an operon, characteristic of specific induction and repression, would not be observed with respect to inhibition by glucose.

### III. REGULATION AND DIFFERENTIATION IN HIGHER ORGANISMS

#### 1. GENERAL REMARKS

The regulatory problems posed by (or to) differentiated organisms are not only of an order of a complexity immeasurably greater than in microorganisms, they are of a different nature. Higher organisms may therefore be expected to possess certain types of cellular regulatory mechanisms which are not found in microorganisms. On the other hand, it seems very unlikely that the main mechanisms recognized in lower forms: allosteric inhibition, induction and repression, should not be used also in differentiated organisms. But it is clear that these mechanisms, by their very nature, can be adapted to widely different situations, and would serve entirely different purposes in *E. coli* and Man, respectively. As we have already pointed out, the specificity of allosteric inhibition, as well as the specificity of induction and repression is inherently "free", in the sense that it results exclusively from the teleonomic construction of the regulatory system. As it turns out, allosteric inhibitors, inducers, and repressors of bacterial systems are, in general, directly related to, or identical with, metabolites of the pathway which they control. This should be considered to reflect the relatively unsophisticated regulatory requirements of free-living unicellular organisms, whose only problems are to preserve their intracellular homeostatic state while adapting rapidly to the chemical challenge of changing environments, and whose success in selection depends on a *single* parameter: the rate of multiplication. Tissue cells of higher organisms are faced with entirely different problems. Intercellular (and not only intracellular) coordination within tissues or between different organs, to insure survival



and reproduction of the organism, becomes a major factor in selection, while the environment of individual cells is largely stabilized, eliminating to a large degree the requirements for rapid and extensive adaptability.

## 2. Nutritional adaptation

These rather obvious *a priori* considerations may perhaps account in part for the somewhat discouraging results which seem to have been obtained so far in attempts to demonstrate induction by substrate or repression by metabolites of enzyme systems in various tissues. Several reports at the conference did illustrate the fact that the level of liver enzymes may vary greatly, depending on the type of diet to which the animals are submitted. But these reports have also illustrated the difficulties of analyzing the mechanisms involved. As Hiatt (see this Symposium, page 367) and also Feigelson, (unpublished) have pointed out, it may be that some of these effects are due to simple stabilization of the enzyme by substrate, rather than to control of their rate of synthesis. Simple stabilization, admittedly, is not a very exciting mechanism. It may well be a physiologically significant one, especially in the liver. The microorganisms have a simple way of getting rid of an enzyme-protein for which there is no more inducer-substrate; they only need to outgrow the protein which has ceased to be synthesized. This simple device is not available to liver cells, and this may justify the selection of the apparently wasteful method of synthesizing enzymes which are stable only in presence of their substrate. It should be added however that many of the systems described here would be difficult to interpret on this basis alone; and one feels confident, in spite of the lack of formal proof, that true induction and/or repression plays an important role in nutritional adaptation of higher organisms.

In any case, it seems clear that nutritional adaptation is not the most important, nor perhaps the most fruitful, field for the investigation of regulatory effects in higher organisms. The development and functioning of these differentiated cellular populations poses three major problems which have hardly begun to be solved at the biochemical and genetic level, namely, differentiation itself, the control of cellular multiplication, and the mechanism of hormone action. Although these three problems are intimately related, we will discuss them separately.

## 3. POSSIBLE MECHANISMS OF HORMONE ACTION

As we have already seen, there are now several recognized cases of "molecular conversion" where a natural hormone appears to be involved, directly or indirectly. Although it is not clear to what extent these particular effects may account for the physiological action of the hormones in question, the suggestion is

that many hormones may act primarily by similar mechanisms. The fact that such mechanisms have not been observed, so far, in bacteria may possibly be significant. It may be recalled that the bacteria, alone among all other forms of life, do not synthesize any steroid. It may also be remarked that an unknown, probably very large, number of microbiologists have at one time or another hopefully added steroids (or adrenalin or insulin) to their bacterial cultures, without ever observing any effect (except catabolic reactions). One is led to wonder whether not only the compounds themselves, but also the type of regulatory mechanism which they control may not be a privilege of differentiated organisms. It would be very unwise however to base such a conclusion on such scanty evidence. And it is to be hoped that, in future years, systematic attempts will be made to verify whether or not certain hormones may not actually act as allosteric inhibitors, inducers, or repressors of certain enzyme systems. The main difficulty of this research will be that no guiding *chemical* principle (based on steric analogy, reactivity, etc.) will help the investigator in the selection of which enzyme systems to test, since again the specificity of induction-repression and of allosteric inhibition is apparently completely independent of the structure and specificity of the controlled enzyme itself. Also, and for the same reasons, it is quite possible that the same hormone may prove to act on different systems, if not by different mechanisms, in different tissues.

## 4. DIFFERENTIATION

It may be in the interpretation and analysis of differentiation that the new concepts derived from the study of microorganisms will prove of the greatest value. One point at least already seems to be quite clear: namely that biochemical differentiation (reversible or not) of cells carrying an identical genome, does not constitute a "paradox", as it appeared to do for many years, to both embryologists and geneticists.

This point may require some elaboration. The control mechanisms discovered in microorganisms govern the *expression* of genetic potentialities. Most of the actual systems however are entirely reversible, in the sense that the effects of inhibitors, inducers, or repressors do not survive for any length of time after elimination of the active agent, and the cells soon return to their initial state.

Differentiation, on the other hand, is stable, and persists once it has been induced. Whether differentiation is ever *completely* irreversible (except in non-growing cells), is an exceedingly difficult question, because the experimental operations which might decide this issue generally cannot be performed. In any case, we need not go into this discussion; let us consider that differentiation may be more or less stable, even attaining irreversibility in some cases. It might then be argued

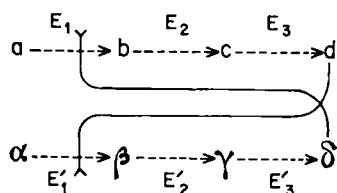


FIGURE 1. Model I. The reactions along the two pathways  $a \rightarrow b \rightarrow c \rightarrow d$ , and  $\alpha \rightarrow \beta \rightarrow \gamma \rightarrow \delta$ , are catalyzed by enzymes  $E_1, E_2, E_3$  and  $E'_1, E'_2, E'_3$ . Enzyme  $E_1$  is inhibited by  $\delta$ , the product of the other pathway. Conversely, enzyme  $E'_1$  is inhibited by metabolite  $d$ , produced by the first pathway.

that since the microbial systems are completely reversible, similar mechanisms could not account for stable differentiation. But it should be clear that the microbial systems must have been geared precisely for reversibility, since selection, in microorganisms, will necessarily favor the most rapid response to any change of environment. Moreover, it is obvious from the analysis of these mechanisms that their known elements could be connected into a wide variety of "circuits", endowed with any desired degree of stability. In order to illustrate some of these possibilities, let us study a certain number of theoretical model systems in which we shall use only the controlling elements known to exist in bacteria, interconnected however in an arbitrary manner.

Consider for instance the following model, which uses the properties of the allosteric inhibition effect, assuming two independent metabolic pathways, giving rise to metabolites  $a, b, c, d$ , and  $\alpha, \beta, \gamma, \delta$  (Fig. 1). Assume that the enzymes catalyzing the first reaction in each pathway are inhibited by the final product of the *other* pathway. By such "crossfeedback" a system of alternative stable states is created where one of the two pathways, provided it once had a head-start or a temporary metabolic advantage, will permanently inhibit the other. Switching of one pathway to the other could be accomplished by a variety of methods, for instance by inhibiting temporarily any one of the enzymes of the active pathway. It should be noted that a model formally identical with this one was proposed by Delbrück (1949) (long before feedback inhibition was discovered) to account for certain alternative steady-states found in ciliates.

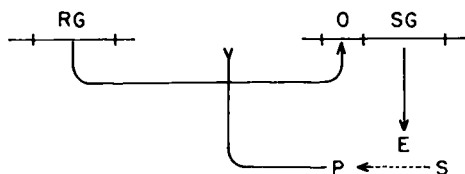


FIGURE 2. Model II. Synthesis of enzyme  $E$ , genetically determined by the structural gene  $SG$  is blocked by the repressor synthesized by the regulator gene  $RG$ . The product  $P$  of the reaction catalyzed by enzyme  $E$  acts as an inducer of the system by inactivating the repressor.

The following model corresponds to a classical induction system, with the only specific assumption that the active inducer is not the substrate, but the *product* of the controlled enzyme. (Fig. 2). Such a system is autocatalytic and self-sustaining. Although it is not self-reproducing in the genetic sense, it should mimic certain properties of genetic elements. In the absence of any exogenous inducing agent, the enzyme will not be synthesized unless already present, when it will maintain itself indefinitely. When the system is locked, temporary contact with an inducer will unlock it permanently. Actually, certain inducible permease systems in *E. coli* may be described in this way, and behave accordingly, as shown by Novick and Weiner (1959), and by Cohn and Horibata (1959). A similar mechanism appears to account for the so-called "slow adaptation" of yeast to galactose, without having recourse to some kind of "plasmagene" as previously believed by Spiegelman (1951).

Two different inducible or repressible systems may be interconnected by assuming that each one produces the metabolic repressor or the inducer of the other. In the first case, as illustrated below (Fig. 3) the enzymes would be mutually exclusive. The presence of one would permanently block the synthesis of the other. Switching from one state to the other could be accomplished by eliminating temporarily the substrate of the live system. In the second case, which may be represented as shown in Fig. 4, the two enzymes would be mutually dependent; one could not be synthesized in the absence of the other, although of course they might function in apparently unrelated pathways. Temporary inhibition of one of the enzymes, or elimination of its substrate, would eventually result in the permanent suppression of both.

In the preceding models, the systems were interconnected by assuming that the metabolic product of one is an inducer or a repressor of the other. Another type of interconnection, independent of metabolic activity,

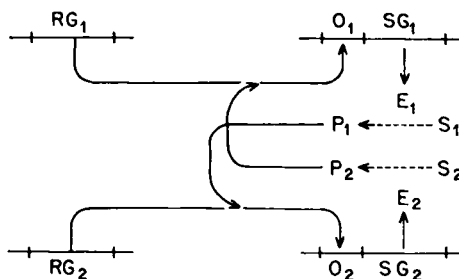


FIGURE 3. Model III. Synthesis of enzyme  $E_1$ , genetically determined by the structural gene  $SG_1$ , is regulated by the regulator gene  $RG_1$ . Synthesis of enzyme  $E_2$ , genetically determined by the structural gene  $SG_2$  is regulated by the regulator gene  $RG_2$ . The product  $P_1$  of the reaction catalyzed by enzyme  $E_1$  acts as corepressor in the regulation system of enzyme  $E_2$ . The product  $P_2$  of the reaction catalyzed by enzyme  $E_2$  acts as corepressor in the regulation system of enzyme  $E_1$ .

would be obtained by assuming a regulator gene controlled by an operator, sensitive to another regulator. For instance, in the system shown below (Fig. 5) a regulator gene controls the synthesis of enzymes within an operon which includes another regulator gene acting upon the operator to which the first one is attached. Such a system would be completely independent of the actual metabolic activity of the enzymes, and could be switched from the inactive to the active state by transient contact with a specific inducer, produced for instance only by another tissue. Once activated, the system could not be switched back except by addition of the aporepressor made by the first regulator gene. The change of state would therefore be virtually irreversible. It is easy to see that, conversely, starting from the active state, transient contact with an inducer acting on the product of  $RG_2$  would switch the system, permanently, to the inactive state.

Finally the following type of circuit might be interesting to consider in relation to cyclic phenomena. In this circuit, the product of one enzyme is an inducer of the other system while the product of the second enzyme is a corepressor (Fig. 6). A study of the properties of this circuit will show that, provided adequate time constants are chosen for the decay of each enzyme and of its product, the system will oscillate from one state to the other.

These examples should suffice to show that, by the use of the principles which they illustrate, any number of systems may be interconnected into regulatory circuits endowed with virtually any desired property. The essential point about the imaginary circuits which we examined, is that their elements are not imaginary. The particular properties of each circuit are obtained only by assuming the proper type of specific interconnection. Such assumptions are freely permitted since, as we have already seen, the specificity of induction-repression and of allosteric inhibition is not re-

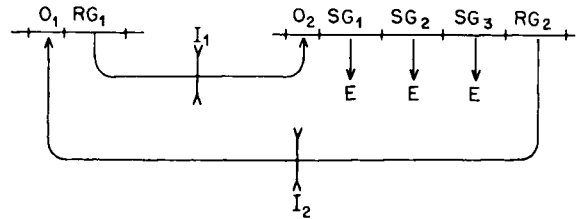


FIGURE 5. Model V. The regulator gene  $RG_1$  controls the activity of an operon containing three structural genes ( $SG_1$ ,  $SG_2$ ,  $SG_3$ ) and another regulator gene  $RG_2$ . The regulator gene  $RG_1$  itself belongs to another operon sensitive to the repressor synthesized by  $RG_2$ . The action of  $RG_1$  can be antagonized by an inducer  $I_1$ , which activates  $SG_1$ ,  $SG_2$ ,  $SG_3$  and  $RG_2$  (and therefore inactivates  $RG_1$ ). The action of  $RG_2$  can be antagonized by an inducer  $I_2$  which activates  $RG_1$  (and therefore inactivates the systems  $SG_1$ ,  $SG_2$ ,  $SG_3$  and  $RG_2$ ).

stricted by any chemical principle of analogy, and apparently is *exclusively* the result of selection for the most efficient regulation.

The models involving only metabolic steady-states maintained by allosteric effects are insufficient to account for differentiation, which must involve directed alterations in the capacity of individual cells to *synthesize* specific proteins. Such models would seem to be most adequate to account for the almost instantaneous, and thereafter more or less permanent, "memorization" by cells of a chemical event. The problem of memory itself might usefully be considered from this point of view.

It has long been recognized, by embryologists and biochemists alike, that "enzymatic adaptation" might offer an experimental approach toward the interpretation of differentiation. The realization that induction and repression are governed by specialized regulatory genes, that both eventually operate by controlling negatively the activity of structural genes, and that the specificity of inducers or repressors is entirely

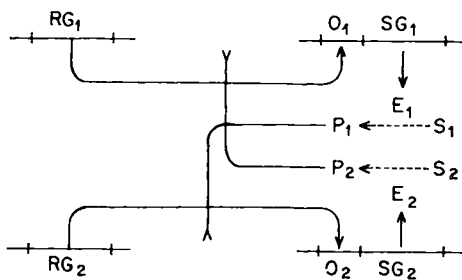


FIGURE 4. Model IV. Synthesis of enzyme  $E_1$ , genetically determined by the structural gene  $SG_1$  is blocked by the repressor synthesized by the regulator gene  $RG_1$ . Synthesis of another enzyme  $E_2$ , controlled by structural gene  $SG_2$  is blocked by another repressor synthesized by regulator gene  $RG_2$ . The product  $P_1$  of the reaction catalyzed by enzyme  $E_1$  acts as an inducer for the synthesis of enzyme  $E_2$  and the product  $P_2$  of the reactions catalyzed by enzyme  $E_2$  acts as an inducer for the synthesis of enzyme  $E_1$ .

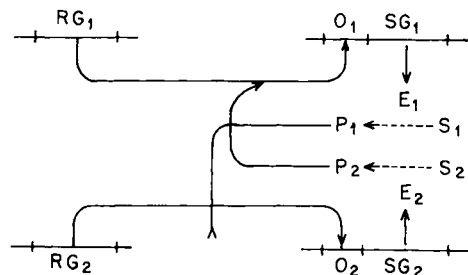


FIGURE 6. Model VI. Synthesis of enzyme  $E_1$ , genetically determined by the structural gene  $SG_1$ , is blocked by the repressor synthesized by the regulator gene  $RG_1$ . Synthesis of another enzyme  $E_2$ , controlled by structural gene  $SG_2$ , is blocked by another repressor synthesized by regulator gene  $RG_2$ . The product  $P_1$  of the reaction catalyzed by enzyme  $E_1$  acts as an inducer for the synthesis of enzyme  $E_2$  while the product  $P_2$  of the reaction catalyzed by enzyme  $E_2$  acts as a corepressor for the synthesis of enzyme  $E_1$ .

*suigeneris*, allows, as we have just seen, the construction of models capable, in principle, of accounting for virtually any type of differentiation. The fact that these mechanisms are not only genetically controlled, but operate directly at the genetic level, and may be in some cases quite independent of any metabolic event in the cell itself, is evidently of special value, since the transitions of state in such systems should very closely mimic true transmissible alterations of the genetic material itself. That differentiation involves induced, specific, and permanent alterations of the genetic information of somatic cells has often been proposed as the only possible interpretation of the "paradox". It should be clear that this type of hypothesis, which meets with almost insuperable difficulties, is in fact completely unnecessary (except perhaps in certain exceptional cases, such as that of the reticulocytes and red cells), since as we have seen the transcription of a gene, not only in a cell, but in a whole cell lineage, may be permanently repressed, or derepressed, depending on an initial, transient event, which would not involve any alteration of the information carried by the gene. And it might be noted that this type of interpretation would not, in any way, be incompatible with the beautiful experiments of Briggs and King (1955) which showed that the nuclei of certain embryonic tissues, in the frog, had lost certain potentialities of expression possessed by the original nucleus of the egg.

The microbial systems actually offer some examples of irreversible effects resulting from repression or derepression. For instance, both lysogenization by an infecting temperate phage, and induction of a lysogenic bacterium, are irreversible consequences of transient conditions favoring, in the first instance, the establishment of a permanent state of self-repression, and in the second a release of the repressed condition. In the repressed (prophage) state which is maintained indefinitely in the absence of inducing agents, the viral genes are inactive in transcription; they are fully active in the vegetative state. Yet the transition from one to the other does not involve any alteration of the information contained in the genetic material of the phage.

The lysogenic systems may also be of some use in thinking about the problem of the control of cellular multiplication. In the prophage (i.e., repressed) state, the phage DNA replicates synchronously with the host cell DNA. In the derepressed state, it replicates about 20 times as fast. The presence of the repressor cannot, by itself, account for this difference. But it is a fact that the decision between synchronous or "wild" replication depends initially upon the regulator-operator interaction. It is most probable that in tissue cells the regulation of multiplication is very complex, since it must simultaneously control several systems which have to be kept in pace. And it may be of some interest to note that even relatively simple regulatory

systems may go astray in several different ways. We know for instance that the constitutive state may be obtained by mutation of either the regulator or the operator. In a system such as the one shown in Fig. 6, mutations of either one of the two operators, or of one of the regulator genes, would abolish the repressive control, resulting either in a constitutive or in a "super-repressed" phenotype. In addition even *temporary* inactivation of one of the loci (for instance by reversible lesions such as are known to be produced by UV light) or temporary blocking of one of the repressors by a complexing agent, would lead precisely to the same permanent phenotypes, which might or might not be reversible by an inducer, depending upon the specific properties of the system. Only by a very thorough genetic and biochemical analysis of such a system could one decide whether the transition was brought about by true mutation, or by temporary inactivation.

These observations may have some bearings on the problem of the initial event leading to malignancy. Malignant cells have lost sensitivity to the conditions which control multiplication in normal tissues. That the disorder is genetic cannot be doubted. That, following an initial event, mutations within the cellular population are progressively selected, leading towards greater independence, i.e., heightened malignancy, is now quite clear, due in particular to the work of Klein and Klein (1958). But while the initial event, responsible for setting up the new selective relationships, may of course be a genetic mutation, it might also be brought by the transient action of an agent capable of complexing or inactivating *temporarily* a genetic locus, or a repressor, involved in the control of multiplication. It is clear that a wide variety of agents, from viruses to carcinogenes, might be responsible for such an initial event.

As a conclusion to this discussion of theoretical models, one would like to turn to experimental examples, and see whether they might, or might not, fit with the interpretations. Unfortunately, in the face of formidable technical difficulties, the study of differentiation either from the genetic or from the biochemical point of view has not attained a state which would allow any detailed comparison of theory with experiment. This is our excuse for using microbial systems as models for the interpretation of differentiation. Eventually, however, differentiation will have to be studied in differentiated cells. The remarkable advances achieved in the methodology of cell cultures encourage optimism. The greatest obstacle is the impossibility of performing genetic analysis, without which there is no hope of ever dissecting out the mechanisms of differentiation. But it should be noted that actual genetic mapping may not necessarily be required. Adequate techniques of nuclear transfer, combined with systematic studies of possible inducing or

repressing agents, and with the isolation of regulatory mutants, may conceivably open the way to the experimental analysis of differentiation at the genetic-biochemical level.

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## DISCUSSION

UMBARGER: Although I have a right to object to only one-third of the term, "NSU," I should like to offer one more plug for my suggestion that the word "end-product inhibition" be employed as an operational term for examples of the endproduct of a biosynthetic inhibiting an early step in its own biosynthetic pathway. Like "repression," the term can be used to describe an empirical observation and should subsequent study so indicate, it can be further described as a feedback mechanism. Should the inhibitory interaction have no such physiological consequence, the operational term is still appropriate.