man could increase by taking medicines prepared from cinnabar.

Isolation of a Repressor Molecule

from a Correspondent in Biochemical Genetics

In 1961 Jacob and Monod proposed a scheme to explain how the activity of genes might be controlled. Their theory was constructed from observations on the lactose operon of *E. coli*, a cluster of three genes which code for three enzymes, two of which are necessary for the utilization of the sugar lactose and other β -galactosides. When wild type *E. coli* is grown with glycerol as a carbon source, all three enzymes are present only in very small amounts. Addition of β -galactosides and, in particular, a non-utilizable β -thiogalactoside, induces a high rate of synthesis of these enzymes. There are at least two regions on the *E. coli* chromosome concerned with the regulation of the lactose operon—a regulator gene and an operator region. Jacob and Monod proposed that the regulator gene codes for a regulator molecule, or repressor, and that this combines with the operator region adjacent to the three structural genes so as to prevent the reading of the operon. The repressor molecules were also supposed to recognize β -galactosides with the result that they were removed from the operator region by the formation of a β -galactoside—repressor complex. This sequence of events would now allow the expression of the lactose operon, probably by permitting the synthesis of a messenger RNA.

The regulator gene has recently been shown to have properties which show that the repressor is a protein molecule. Insight into the molecular mechanism of regulation has, however, rested only on indirect experiments because it has not been possible to detect and assay repressor molecules in the test-tube. The whole status of the field has now been altered by the recent work of Professor Walter Gilbert and Dr. Benno Müller-Hill of Harvard University, who have detected the repressor molecule for the lactose operon (*Proc. U.S. Nat. Acad. Sci.*, **56**, 1891; 1966). Their assay depends on the ability of the repressor to bind an inducer β -galactoside. Using a radioactive inducer, they showed by equilibrium dialysis that more inducer was found inside the dialysis bag containing a cell extract than outside the bag. Control experiments on extracts from cells which contain a deletion of the regulator gene show that these do not bind radioactive inducer. They have shown that this repressor is a protein molecule with a probable molecular weight of about 150,000–200,000, and that there are about ten molecules of repressor per gene copy in *E. coli*.

This is the first and crucial step towards the biochemical study of the control of gene expression.

Enzyme Mechanisms

from a Correspondent in Molecular Biology

THE power of relaxation methods for the measurement of fast chemical processes is nowhere more spectacularly revealed than in the temperature-jump studies of enzyme kinetics which have been emerging from a number of laboratories. This month a further study on ribonuclease throws new light on the mechanism of

the enzyme, and an important article from the laboratory of Eigen goes some way towards defining the characteristics of an allosteric system of interacting sites.

Erman and Hammes (*J. Amer. Chem. Soc.*, **88**, 5607 and 5614; 1966) have used a stopped flow temperature-jump technique to study the interaction of both cytidine 2':3'-cyclic phosphate and cytidylyl-3':5'-cytidine with ribonuclease. In this system the usual temperature-jump method fails because the equilibrium is overwhelmingly biased in favour of the product of hydrolysis—cytidine 3'-phosphate—and its complexes with the enzyme. By introduction of the stopped flow, the temperature jump can be applied 16 msec after the mixing of enzyme and substrate. The system is found to exhibit two relaxation processes, one independent of concentration at high excess of substrate, the other dependent on it. These are attributed respectively to an isomerization in the enzyme-substrate complex and to the binding of the substrate to the enzyme. Taken together with earlier work on the interaction with cytidine 3'-phosphate, a mechanism for the hydrolysis of the substrate has been evolved which appears to account for all relaxation, equilibrium and steady-state data. Moreover, the pH-dependence of the relaxation processes is interpreted as indicating the existence of five states of the enzyme-substrate complex, involving three or more ionizing groups in the enzyme, with specified pK 's. Parallel reaction paths are also inferred.

In the accompanying article, the same authors consider the ribonuclease-cytidylyl-3':5'-cytidine system, which is shown to have similar characteristics. It is recognized that there is at present no means whereby the formalism relating the states of the system can be interpreted structurally. To the extent that certain groups, among them two histidines, are believed to be present in the active centre, a number of speculations are possible, but this is another case in which the results of crystallographic analysis of the protein are eagerly awaited.

An article by Kirschner *et al.* (*Proc. U.S. Nat. Acad. Sci.*, **56**, 1661; 1966) examines the binding of NAD to glyceraldehyde-3-phosphate dehydrogenase, an enzyme known to contain four identical sub-units. The system was selected because it was believed to represent a tractable case of "homotropic" ligand binding. Three relaxation processes in substantially different time ranges were observed, so that the dependence of each step on concentration could be determined. If it is assumed that conformational transitions are slow in comparison with bimolecular binding processes, the three relaxation times can be used to derive rate constants. The kinetics can be very satisfactorily accounted for in terms of the postulates of the appropriate allosteric hypothesis—that the apoenzyme exists only in two tetrameric modifications and that the intrinsic dissociation constants of the binding sites are identical in both forms. It is also shown that there is a high degree of co-operative (all-or-none) behaviour between the sub-units. These results can evidently be most simply explained in terms of the allosteric theory, for which they may be regarded as providing support. As the authors point out, it will be of considerable interest to obtain similar information on a range of other sub-unit enzymes, and to see which generalizations appear to survive.