Biological Control Mechanisms

Contemporary biological research is strongly linked with biochemistry, but for a comprehensive understanding of living organisms, the discovery of enzymes, of metabolic pathways, and of the chemical nature of the genetic material must be followed by studies on the mechanism by which the production and action of biological catalysts are regulated. The existence of control mechanisms is indicated by the rapid adjustments which microorganisms make to their environment and by the homeostatic systems employed by animals to make adjustments in the environment of their cells. Moreover, it is apparent that there exists in organisms competition among physiological processes for common metabolites and enzymes, as well as selective permeability barriers, and a host of very specific metabolic regulators. To disseminate recent findings in this area of research and to stimulate broader consideration of these problems, a symposium on New Concepts Regarding Biological Control Mechanisms was sponsored by Section N (Medical Sciences) at the annual AAAS meeting in Philadelphia on 27 and 29 December. There were half-day sessions on (i) repression, (ii) feedback control of enzyme action, (iii) hormonal phenomena, and (iv) transport across cell membranes. The respective chairmen were B. Magasanik, H. E. Umbarger, E. R. Sutherland, and C. R. Park.

Investigation of bacterial physiology has recently contributed new concepts that will undoubtedly find extensive application in studies of higher organisms. These concepts are repression, or the inhibition of the formation of one or more enzymes of a biosynthetic pathway by an end-product of the pathway, and negative feedback, or end-product inhibition of an early reaction of a biosynthetic pathway. During the discussion of these phenomena, it was suggested by B. Ames that feedback is the means for fine control of biosynthetic pathways, whereas repression may be the mechanism for coarse control. It was made clear by L. Gorini and by B. Magasanik that enzyme induction can now be interpreted as the derepression of an enzyme-forming mechanism of an organism. Genetic change releasing the repression makes induction unnecessary. It is postulated that repression results from the combined action of an aporepressor (a macromolecule?) plus a corepressor (small metabolite?); the inducer perhaps prevents the formation of the active repressor. Alternatively, the inducer may actually combine with and inactivate the repressor. Gorini illustrated his comments by reference to studies on the regulation of the formation of ornithine transcarbamylase.

Since there are many examples of repression by glucose, Magasanik has studied the relationship between the system affected by an inducer and that affected by glucose. He based his study on previous findings of others that indicated an inducer for β-galactosidase in *E. coli* first stimulates the formation of messenger RNA, which in turn can then promote enzyme formation in the absence of the inducer. He was able to show that, since both glycerol (a glucose catabolite) and fluorouracil (an inhibitor of the formation of active messenger RNA) are effective as repressors only during the 4-minute period when specific RNA is being formed, induction and catabolite repression apparently affect the same step in enzyme biosynthesis, namely, the production of the specific messenger RNA required for the synthesis of the enzyme.

The histidine biosynthetic pathway of microorganisms has provided an excellent system for the study of both feedback and repression. Ames discussed his studies on the simultaneous repression (coordinate repression) of enzymes of the pathway. The data point to the action of an operator gene, such as that postulated by Jacob and Monod for β-galactosidase, which turns on and off a group of structural genes and whose position on the genome may be inferred from the "polarity" of the effects on the enzymes affected.

The versatility of *E. coli* in adjusting its metabolic machinery to different carbon sources has been extensively investigated by H. Kornberg. He showed how this organism alters its enzymatic make-up (for example, isocitratase and malate synthetase for the glyoxylate cycle, condensing enzyme for the tricarboxylic acid cycle, glyoxylate carboligase for glycollate utilization), depending upon the nutrients offered. It was suggested that these phenotypic change are due to derepression.

H. E. Umbarger called attention to the pioneering studies and postulations on the phenomenon of end-product inhibition (negative feedback). It is certainly an indication of the rapid acceptance of the concept and of its broad application that his review necessarily began with studies reported in 1950! G. N. Cohen discussed the biosynthesis of several amino acids that share portions of their biosynthetic pathways. These studies are important because, when a certain early reaction sensitive to end-product inhibition is required for the formation of two amino acids whose biosynthetic routes have diverged from a common pathway, one may wonder whether negative feedback by one end-product may not shut down the production of both end-products. In a clearly delineated system, namely, the phosphorylation of aspartic acid necessary for the production of both lysine and threonine, each of the two amino acid end-products is capable of only moderately inhibiting the required kinase reaction. Cohen showed that the limited inhibition occurs because there are two distinct kinases, one inhibited by lysine but not by threonine, the other by threonine but not by lysine. This work is of major importance for an understanding of control in branched biosynthetic pathways. However, there is some evidence that multiplicity of enzymes that carry out the same reaction may not always be the means for avoiding the complete inhibition of a reaction shared by two or more biosynthetic routes.

The mechanism by which an end-product inhibits an enzyme on its biosynthetic pathway may be nearing explanation. A. Pardee reported that crystalline aspartic carbamylase appar-

ently has a second site (in addition to that effecting its characteristic catalytic action) at which cytidine triphosphate acts. This substance, the end-product of the pathway, may deform the enzyme molecule by combining at the second site (allosteric site) and thus influence the binding of the substrate, aspartic acid. Selective destruction of the second site can be accomplished by various treatments. The CTP binding is apparently dependent on the existence of the tetramer enzyme complex.

H. S. Moyed discussed the intriguing action of the antibiotic Psicofuranine in inhibiting xanthylic acid aminase, the enzyme catalyzing the terminal step in the synthesis of guanylic acid. He suggested the possibility that this agent might be acting in a manner similar to that of a natural inhibitor of the enzyme system.

While microorganisms generally offer the simplest and therefore the most useful experimental systems for disclosing fundamental control mechanisms, animal investigations are important not only for comparative purposes and for the elucidation of complex physiological processes but also because mechanisms exist which are more important to, or actually unique in, higher species. Hormonal regulation of metabolism is an example of metabolic control of special importance in animals. A hormone whose physiological action is at least partly explainable on an enzymatic basis is epinephrine. Sutherland and his colleagues have shown that this hormone will enhance the formation of cyclic-3′,5′-adenylic acid, a cofactor in the activation of phosphorylase, which is the enzyme required for the breakdown of glycogen. This unusual nucleotide is produced from adenosine 5′-phosphate by a cyclase that is present in the "nuclear" fraction of liver homogenate but is believed to be in the cell membrane. Sutherland and Rall reported on extensive studies of the metabolism and physiological effects of the substance. It is of considerable interest that the highest concentration is found in brain.

In a report not specifically dealing with hormones but carrying major implications regarding the control of enzyme action, O. H. Lowry discussed his recent findings that glycolysis may be controlled by inhibition of phosphofructokinase by ATP and suggested that there may be two inhibitory sites for ATP. A number of metabolites are able to overcome this inhibition; fructose diphosphate and cyclic-3′,5′-AMP are most potent in this regard. The situation is complicated by the fact that combinations of certain metabolites may exert unusually large inhibitions, and the kinetics of the reaction are difficult to interpret. Bacterial phosphofructokinases respond differently to inhibitors and seem to be under more limited control than the mammalian enzyme.

The ability of hormones to alter enzyme activity by influencing the association of enzyme molecules has been well demonstrated by G. Tomkins, who discussed the response of glutamic dehydrogenase to steroids. It is now clear that this enzyme can exist in aggregates consisting of different numbers of individual protein molecules. The state of aggregation is influenced by the concentration of steroids or other metabolites, while the binding of substrates is, in turn, determined by the state of aggregation. Steroids of proper structure cause disaggregation into subunits which show decreased glutamic dehydrogenase activity but enhanced alanine dehydrogenase activity. The aggregate has a molecular weight of one million while the subunits have a molecular weight of 250,000. These subunits are in turn composed of smaller units of perhaps 40,000 to 50,000 molecular weight. There is no definitive evidence as yet which indicates that the effects of steroid hormones on glutamic dehydrogenase play any significant physiological role.

This mechanism of steroid regulation of enzyme activity is not a universal one; there are now examples in which alterations in molecular weight of the enzymes do not occur. Physical measurements suggest that the configuration of the protein molecules may be influenced by steroids. Moreover, there is new work indicating that the hydrocortisone-induced elevation of liver enzymes involves, in part, the hormonal stimulation of RNA synthesis. Still another type of hormonal activity is the stimulation of transhydrogenase reactions by steroids. Undoubtedly it will eventually be shown that a variety of mechanisms are involved in the physiological action of the many types of hormones present in animals. A continuing problem will be the evaluation of the results of isolated enzyme studies in terms of the metabolism of the intact animal.

The regulation of metabolic pathways by alterations in the production or state of enzymes depends of course on the actual entrance of metabolites, or of the regulators themselves, into the cell. The final session of the symposium was therefore devoted to the problems of transport across membranes.

As was pointed out by C. R. Park, although there is considerable evidence for the existence of carriers within membranes, attempts to isolate carriers from broken cell preparations have in the past been quite unsuccessful. For this reason there is great interest in the experiments of D. O. Rudin and P. Muller, who are studying the transport problem with artificial membranes. Since the core structural element of cell membranes is thought to be a bilayer of phospholipid molecules, these workers have prepared artificial bimolecular films using lipids isolated from cell membrane material. These films are developed across apertures 1 to 2 cm in diameter in polyethylene sheets immersed in saline solutions. The membranes thus formed separate two aqueous phases and permit transport measurements. They are stable to shock, are self-sealing, and, upon the addition of specific substances, exhibit electrical behavior analogous to that observed in certain natural membranes.

A fundamental phenomenon involving transport is exemplified by the maintenance of differential gradients of sodium and potassium ions on the two sides of cell membranes. J. C. Skou outlined the requirements for a postulated carrier system whose properties are deduced from experimental observations. Since transport of Na^+ and K^+ is a process which derives its energy from the utilization of ATP, he has prepared broken nerve cell membranes and identified the transport system in the fragments by the fact that ATPase (ATP-splitting) activity is stimulated by the addition of Na^+ and K^+ to the medium. This system has also been localized in the cells of other tissues that show active transport of Na^+ and K^+. Although Skou's enzymatic system exists as an organized unit of the disrupted membranes, it represents the closest approach at present to understanding a transport process on a molecular level. Another noteworthy advance is A. Leaf's work on salt and water transport through the wall of the toad bladder, which

may be considered as a relatively simple biological analog of the kidney. The stimulatory effect of the hormone vasopressin on the transport of Na^+ requires that the hormone be on the serosal side of the membrane in spite of the fact that the rate-limiting step apparently occurs in the mucosal barrier. Similar experiments may shed light on the role of K^+ in the transport of Na^+.

Because of the great difference in complexity of the organisms under study, it is unlikely that the remarkable progress characteristic of recent microbial investigations will soon be paralleled in studies on animals. Nonetheless, it was apparent from the meeting that the results obtained from microbial studies are rapidly finding application in animal work and that noteworthy advances are being made in elucidating control mechanisms in the higher species.

The program was arranged by D. Stetten, Jr., and O. Touster in collaboration with the session chairmen. The National Institutes of Health awarded a generous grant in support of the symposium.

OSCAR TOUSTER
Department of Biochemistry,
Vanderbilt University,
Nashville, Tennessee

Endocrine Control of Growth

A symposium on growth, sponsored by the Division of Comparative Endocrinology of the American Society of Zoologists, was held on 28 December 1962 in Philadelphia. Interest in comparative endocrinology has steadily increased in the last decade. The Division now has close to 400 members, and attendance at this symposium was large.

Previous work has shown that systems analysis of endocrine control of lower animals might very well be extrapolated to an analysis of endocrine control in the higher vertebrates. A prime example of this is neuroendocrine integration and the structure of neurosecretory cells. Although previous symposia on comparative endocrinology, including three international congresses, have been successfully conducted in recent years, none has been primarily concerned with growth processes. The present symposium had as its aim the discussion of not only those well-known vertebrate hormones that may act on growth but also substances affecting growth in lower animals, and even plants, where the classical definition of a hormone could not possibly apply. In addition, speakers invited to participate were, in general, established workers in the field who had not presented similar material at the last two international congresses of comparative endocrinology.

The plant growth substances, discussed by Bruce Stowe (Yale), proved to be most stimulating to the zoologists present. Because the indoles are the major auxins, a technique was developed whereby neutral indoles could be separated and identified by gas chromatography with a Versamid 900 column. Since this technique is specific for neutral indoles, any indole acids to be studied must first be converted to neutral products. Since gas chromatography by flame ionization can detect parts per billion, this technique could allow detection of auxins in specific growth regions and perhaps obviate the use of bioassays. Although the structure of some gibberellins is now known, their mode of action in restoring normal growth to mutant dwarf plants is still an open question. It is now believed that the gibberellins interact with the auxins and kinetin-like substances to bring about homogeneous growth in some plants. A most interesting series of experiments involving the local application of kinetin-like substances to plant leaves was discussed in detail by Stowe. The results indicate that these substances act to mobilize amino acids in the plant and thus result in enhanced protein synthesis. Of great interest was the report that certain lipids enhance the synergistic action of auxin and gibberellic acid in promoting plant growth. Substances such as methyl linoleate have a profound effect when applied with the plant growth substances, and although the reason for this effect is still not known, it is reminiscent of the action of certain lipids in enhancing the effect of insect growth hormones.

The "immortal" hydra was the subject of Burnett's (Western Reserve) discussion. Although hydra is a vigorously polarized animal, grafting experiments have indicated that the polarity can be inverted. A substance is produced in hydra which has the capacity to induce head formation. Assay animals placed on an agar block containing an extract of this material form many heads and in some cases appear to be a mass of heads. Burnett believes that growth in hydra is controlled by both growth-promoting and growth-inhibiting substances, although the presence of two different substances has not been conclusively demonstrated. Burnett argued that the rapidly proliferating hypostomal region of hydra contains neurons that may be neurosecretory. Neurosecretory stains indicate that there are granules in the cell bodies and in the axons. In the discussion following this paper it was pointed out that neurosecretory-like elements have been noted in hydra under the electron microscope. If these observations are confirmed, it will mark the first demonstration of neurosecretion in the Cnideria, a phylum where the occurrence of any type of neuron at all has been a matter of conjecture for a long period of time. Whether the activity of these "neurosecretory cells" is requisite to growth in hydra is not yet known.

Although the basic endocrine control of molting in decapaod crustaceans remains as postulated several years ago, Bliss's (American Museum of Natural History) recent studies have indicated the extreme importance of environmental conditions on the pre-molt (proecdysis) stage. She reported on her recent studies which indicate that photoperiod, moisture, temperature, and the presence or absence of other crustaceans affect the onset of proecdysis. That is, the animal will only enter proecdysis when it is more or less assured that environmental conditions are favorable for surviving those critical days following the shedding of the exoskeleton when it is no longer protected by its hard outer skin. Thus stress situations will prevent the onset of this hormonally-controlled process. The ability of the crustacean to alter its molting cycle in this manner may be one reason for the long survival of this class of animals.