# Meetings

## Enzyme Regulation

Nearly every chemical reaction within living organisms is controlled by enzymes. All enzymes, of which more than 1000 have been discovered, are targets of control mechanisms.

The regulation of enzymes in various tissues was discussed at the Fifth International Symposium on Regulation of Enzyme Activity and Syntheses in Mammalian Tissues, held at Indiana University School of Medicine, Indianapolis, 3–4 October 1966. Scientists from France, Germany, Great Britain, Italy, Japan, and the United States reported their latest findings.

In the opening session on enzyme regulation and coenzyme metabolism, Britton Chance (University of Pennsylvania) reported on the responses of the cytosol to mitochondrial substrates. The technique developed in his laboratories combines microfluorometric observation with microinjection of appropriate substrates and cofactors into localized portions of ascites tumor cells grown on glass. Experimental evidence was obtained for a malate dehydrogenase activity of the cytosol of these ascites cells of sufficient magnitude to cause a considerable displacement of the NADH level. The magnitude of the displacement was commensurate with that found on addition of glyceraldehyde-3-phosphate. Chance demonstrated not only the existence of the malate dehydrogenase in the cytosol, but also its potentiality to alter significantly the redox state of the cytosol. Studies on the biosynthesis of NAD by a direct method of tracing metabolism in vivo were discussed by Osamu Hayaishi (Kyoto University, Kyoto, Japan). Radioactive substrates were injected directly into the portal vein of mice, then analyses were made of the isotopically labeled metabolites in the liver. When administered in small doses, nicotinic acid was a better precursor of liver NAD than was nicotinamide. When given in large doses, however, nicotinamide was a much better precursor of NAD than was nicotinic acid. Analyses of the distribution of $C^{14}$ in various organs indicated that a large portion of nicotinamide-$C^{14}$ was first excreted from liver, accumulated in the gastrointestinal tract, deamidated to nicotinic acid, reabsorbed into the liver, and served as precursor to NAD over a prolonged period of time.

Enzyme regulation in different organs was described by H. Guy Williams-Ashman (Johns Hopkins University), John N. Fain (Brown University), Nobuhiko Katunuma (Tokushima University, Tokushima, Japan) and Leon Goldstein (Harvard Medical School). Williams-Ashman reported that following a single injection of testosterone propionate into orchiectomized or hypophysectomized rats there occur massive yet transitory changes in the ability of soluble prostatic extracts to catalyze the incorporation of DNA of deoxyribonucleotides derived from deoxyribonucleoside triphosphates. The enzyme activities measured appeared to be catalyzed mainly by a "replicative" type of DNA polymerase that preferentially utilizes single-stranded DNA as primer. In castrated rats given daily injections of testosterone propionate the levels of this soluble prostatic DNA polymerase rise to a maximal level within 3 to 5 days, but decline subsequently, despite the continual administration of the hormone. The role of RNA and protein synthesis in the lipolytic action of growth hormone in isolated fat cells was studied by Fain. He suggested that the acceleration of lipolysis by growth hormone and glucocorticoids may involve DNA-dependent synthesis of RNA, and that the lag period in the action of these hormones may be necessary for growth hormone to stimulate the synthesis of RNA and protein molecules. Katunuma described the discovery, isolation, and purification of glutaminase isozymes in brain, liver, and kidney of the rat. He suggested that the organ-specific differences in inducibility of glutaminase isozymes were analogous to differences in their physicochemical properties, such as their optimal *p*H values and heat stability. The regulation of ammonia production in rat kidney, with particular reference to the role of glutaminase in the control of renal ammonia synthesis, was discussed by Goldstein. Renal glutaminase may be regulated by the level of its product in the kidney. During acidosis the concentration of glutamine fell slightly, ammonia concentration rose, and the level of the activator, phosphate, was unaltered, thus suggesting that these factors do not play a positive role in the adaptation of renal ammonia production during acidosis.

Metabolic regulation through enzyme biosynthesis was discussed by B. Connor Johnson (University of Oklahoma), Harold J. Fallon (University of North Carolina), and Charles G. Smith (The Upjohn Company). Johnson studied the induction of liver glucose-6-phosphate dehydrogenase in the rat. He found that this enzyme is particularly induced by dietary carbohydrate in the presence of adequate protein. The protein synthesis site at which the induction of glucose-6-phosphate dehydrogenase occurs appears to be at the transcription level from DNA to RNA. Fallon presented evidence that diet and hormonal state are significant factors in the control of the synthesis of endogenous serine in rat liver. Restriction of protein intake resulted in a marked increase in hepatic 3-phosphoglycerate dehydrogenase and a lesser rise in phosphoserine phosphatase. However, serine dehydratase decreased on this diet. Studies with actinomycin and cycloheximide suggest that the changes noted in enzyme activity were caused by alterations in enzyme synthesis. Cortisone injections suppressed the level of 3-phosphoglycerate dehydrogenase and D-glycerate dehydrogenase. Smith reported on the various biological and biochemical properties of the antibiotic, tubercidin (7-deaza-adenosine). He noted that the hydrocortisone-induced increase of tryptophan pyrrolase activity in rat liver was blocked by administration of 7-deaza-adenosine, methyl ester of 7-deaza-adenosine 5′-phosphate, or 7-deaza-inosine. It is important that, at drug dosages that clearly inhibited the hydrocortisone-induced enzyme increase, 7-deaza-adenosine and the methyl ester of 7-deaza-adenosine 5′-phosphate did not affect the tryptophan-induced increase in the pyrrolase activity. However, the tryptophan-induced increase in the enzyme activity was blocked by 7-deaza-adenosine. Thus, these compounds are useful in selectively inhibiting the hormone- or substrate-induced increase in hepatic tryptophan pyrrolase activity.

Studies on the role of enzymes in

development were reported by Francois Chapeville (Commissariat a l'Energie Atomique, Gif-sur-Yvette, France) and Fabio Sereni (University of Milan, Milan, Italy). Chapeville described a system catalyzing the synthesis of taurine from inorganic sulfate found in the yolk sac of chick embryo. Several enzymes involved are present only in endodermal cells of the yolk sac; others are present in the yolk sac and in the embryo. The crucial role exerted by birth on activating RNA synthesis in rat liver was emphasized by Sereni. The activation of RNA synthesis leads to an increased synthesis of a selected number of protein enzyme molecules, whose activity rises as soon as the extrauterine life starts. There is a decreased rate of incorporation of pyrimidine precursors into nuclear RNA in newborn rats adrenalectomized shortly after birth. Hydrocortisone does not appreciably influence the rate of liver RNA synthesis in newborn rats. Glucocorticoids, starting from the fourth day of life, interfere with RNA metabolism, mainly stabilizing newly formed RNA molecules.

Masami Suda (Osaka University, Osaka, Japan) and Helmut Holzer (University of Freiburg in Breisgau, Germany) compared enzyme regulation in mammalian and microbial organisms. Suda showed that the unicellular organism, *Micrococcus ureae*, is able to induce the sequential enzyme systems metabolizing tryptophan and mandelic acid. From the reaction of the coinducer in bacterial cells it seemed possible that hormones in higher organisms might act in a similar way by binding to a receptor, thus causing an allosteric change on it and increasing its sensitivity to a metabolic inducer. Suda also reviewed the biophysical, kinetic, and regulatory differences between muscle and liver types of pyruvate kinases in rat. He examined the role of the nervous system in regulation of enzyme activities and discussed the homeostatic role of nutritional, hormonal, and neural regulation at the enzyme level. Holzer described metabolite-induced enzymatic inactivation of glutamine synthetase in *E. coli*. Addition of ammonium salts to *E. coli* cells grown on a medium containing no ammonium salts produces an almost complete inactivation of glutamine synthetase within one to two minutes. Addition of ammonium salts to the medium causes not only inactivation of glutamine synthetase, but also repression of the synthesis of the enzyme. Glutamine synthetase inactivated by the addition of ammonium ion to intact cells is reactivated after the ammonium ion is washed out. An inactivation of glutamine synthetase activity with only a small effect on the glutamyl transferase activity was also demonstrated in a cell-free system. Holzer's results support the view that the inactivation produced by ammonium ion in intact cells is identical with the enzymatic inactivation induced by glutamine in the cell-free extract.

Studies on control of gluconeogenesis were presented by Henry A. Lardy (University of Wisconsin), John R. Williamson (University of Pennsylvania), and George Weber (Indiana University). Lardy described the view of gluconeogenesis gained from the rat treated with tryptophan. His results support the concept that aspartate, malate, and, to a minor extent, citrate, are essential intermediates in gluconeogenesis, and that specifically they are the form in which the precursor of phosphoenolpyruvate is transferred across the mitochondrial membrane. Tryptophan was a useful tool for investigating the site of action of agents that affect gluconeogenesis. The injection of glucose greatly depresses the accumulation of aspartate, malate, and citrate in the rat treated with tryptophan. Lardy was able to duplicate in the isolated, perfused rat liver a number of the responses to tryptophan observed in the intact rat. Williamson reported effects of fatty acids, glucagon, and anti-insulin serum on the control of gluconeogenesis and ketogenesis. Fatty acids added to the isolated, perfused rat liver stimulated the rate of gluconeogenesis from 3-carbon precursors. The proposed mechanism for this effect involves activation of pyruvic carboxylase by acetyl-CoA and facilitation of glyceraldehyde-3-phosphate dehydrogenase by NADH. Glucagon stimulated glycogenolysis, lipolysis, and ketogenesis in rats in vivo; Williamson suggested that the glucagon-induced gluconeogenesis was secondary to increased lipolysis. The glucagon effects in normal rats in vivo were transient compared with similar but more pronounced and prolonged effects produced by this hormone in rats treated with anti-insulin serum. Most of the metabolic alterations produced in the anti-insulin serum plus glucagon-treated rats were reversed by insulin. Williamson suggested that the acute results of insulin deficiency are a manifestation of the effects produced by endogenous glucagon and other lipolytic hormones. Weber described the "metabolic switch action" of free fatty acids. He provided evidence for the operation of a feedback mechanism by free fatty acids which may control the overall metabolic direction of gluconeogenesis and glycolysis. He pointed out that the pyruvate kinase is overwhelmingly more active than the opposing enzymes of gluconeogenesis. Because of this unfavorable ratio at the early step of gluconeogenesis, he assumed that mechanisms existed by which pyruvate kinase might be acutely inhibited, which would then prevent recycling and permit the operation of gluconeogenesis. In view of the earlier work of Krebs *et al.* and that of Haynes in liver slices and the reports of Ashmore *et al.*, Cahill *et al.*, and Weinhouse, and the new, extensive data of Williamson showing that addition of fatty acids increased gluconeogenesis, Weber examined the effects of fatty acids at the enzyme level. He reported that the key enzymes of glycolysis, glucokinase, phosphofructokinase, and pyruvate kinase were inhibited by physiological concentrations of fatty acids. Since in gluconeogenic conditions (diabetes, starvation, or steroid treatments), high plasma-free fatty acids occur, Weber suggested that fatty acids may function physiologically in acute adaptation as a metabolic directional switch.

The action of hormones in vitro was investigated by Van R. Potter (University of Wisconsin) and Abraham White (Albert Einstein College of Medicine). Potter showed that there are multiple factors involved in the regulation of the activity of tyrosine transaminase in whole animals and in tissue culture. The most likely factors so far implicated are cortisone, insulin, and tryptophan and its metabolites. White showed that exposure of thymocytes to cortisol caused an inhibition of RNA, DNA, and protein synthesis. He obtained a good correlation between the relative potencies of various steroids in inhibiting uridine incorporation by thymocytes in vitro and their known thymolytic potency in vivo.

Regulation and isozymes and feedback mechanisms were discussed by Robert E. Olson (St. Louis University), Howard M. Katzen (Merck Institute for Therapeutic Research), and Douglas S. Riggs (State University of New York at Buffalo). In reporting studies carried out in vitamin-E deficient rabbits on the regulatory function of vitamin E, Olson suggested that a deficiency of

vitamin E may represent a state of excessive synthesis of catabolic and redundant enzymes which is repressed by vitamin E and inhibited by ethionine. If the hereditary muscular dystrophies are caused by a mutation in a regulatory gene, the similarity of these disorders to vitamin-E deficiency and the ineffectiveness of alpha-tocopherol as a therapeutic agent are explained. Katzen described the nature of function of the multiple forms of mammalian glucose-ATP-6-phosphotransferase (hexokinase). The total rat liver activity is now resolved into four distinct forms. The four enzymes have been separated and partially purified. Although they differ from each other, each distinct hexokinase is uniform in its properties from tissue to tissue, even though present in differing proportions depending upon the tissue source, animal age, and nutritional factors. A fifth type of hexokinase, apparently unique to sperm, was also described. On the basis of a correlation between the tissue distribution of the multiple forms of hexokinase and the tissues' insulin sensitivities, a hypothesis for the significance of the isoenzymes to the mechanism of action of insulin was outlined. Riggs presented a theoretical paper on the topic: feedback—fundamental relationship or frame of mind? Feedback is one of the four fundamental ways in which variables can be related to each other. Riggs pointed out that there is urgent need for universal agreement about the precise meaning of the term "feedback."

Effects of a liver carcinogen and the actions of radiation were discussed by Harry V. Gelboin (National Cancer Institute) and Olga Greengard (Harvard Medical School). Gelboin reported on the effect of methylcholanthrene, phenobarbital, and aflatoxin on RNA polymerase of rat liver. In the absence and presence of ammonium sulfate, methylcholanthrene and phenobarbital administered in vivo stimulate and aflatoxin markedly decreases RNA polymerase activity in the nuclei of rat liver. Greengard reported a fifteen-fold increase in the activity of tyrosine transaminase by the in vivo synergistic action of glucagon and hydrocortisone. She found that irradiation, unlike actinomycin, inhibited the "cofactor-type" inductions of tryptophan pyrrolase and tyrosine transaminase, but did not block the "hormone-type" induction of these enzymes. Greengard also discussed the mechanisms of induction in animals.

As in previous years, the Special Symposium Lecture was delivered by Sir Hans A. Krebs (Oxford University, Oxford, England) who discussed the redox state of diphosphopyridine nucleotides in regulation of metabolic processes. Krebs pointed out that the ratio of lactate to pyruvate reflects the redox state of dinucleotides in the cytoplasm; the glutamate system indicates the position in the mitochondrial matrix and the beta-hydroxybutyrate system that in the mitochondrial crystae. In diabetes the decrease in the ratios of the two mitochondrial systems was contrasted to the increase in the ratio of the cytoplasmic system. However, in starvation all three systems moved in the same direction to approximately the same extent. The ratios were very much lower in the mitochondrial systems than those of the cytoplasm, differing by a factor of 100 in liver from fed or starved animals and by a factor of 20 in the liver of diabetic rats. Krebs pointed out that the fact that calculations for the glutamate and beta-hydroxybutyrate dehydrogenase systems led to the same values for the ratio of NAD to $NADH_2$ implied that the substrates of these two dehydrogenases were in equilibrium with the same NAD-$NADH_2$ pool.

Indiana University honored Sir Hans A. Krebs at the end of his lecture by awarding a citation of the President of the University. Krebs also received the highest award given by the State of Indiana when he was made a Sagamore of the Wabash. In appreciation of his contribution to this Symposium series over the past half decade, volume 5 of *Advances in Enzyme Regulation* is dedicated to Sir Hans A. Krebs.

The symposium was sponsored by Indiana University School of Medicine, the American Cancer Society Institutional Grant, Hoffman-LaRoche, Eli Lilly and Co., Merck Sharp & Dohme, The Upjohn Co., and the Wellcome Co. The full text of the papers, edited by George Weber, will be published in the spring of 1967 as volume 5 of *Advances in Enzyme Regulation* (Pergamon Press, New York and Oxford). Volumes 1 through 4 of this series of Conferences on Enzyme Regulation in Mammalian Systems were published in 1963 through 1966 and presented the proceedings of the previous four symposia.

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