Meetings

Enzyme Regulation in Mammalian Tissues

The regulation of enzyme activity and synthesis in mammalian tissues was the theme of an international symposium held 4–5 October 1965 at Indiana University School of Medicine, Indianapolis. The theories that have been developed on control of enzymes in unicellular organisms are not necessarily applicable to mammalian enzymes, and this meeting, the fourth in a series begun in 1962, afforded an opportunity to discuss and clarify problems unique to enzyme regulation in mammalian organisms. The control of enzymes in liver and other tissues was highlighted as a background to the evaluation of the failure of regulation of enzymes and metabolic pathways in cancerous cells.

The biosynthesis of fatty acids in adipose tissue as related to regulation of lipid metabolism through enzymes was discussed by E. G. Ball (Harvard Medical School) and A. Gellhorn (Columbia University). Ball was concerned with the question of the balance between reduced coenzymes produced and utilized in adipose tissue during lipogenesis. His calculations support a conclusion that reduced nicotinamide adenine dinucleotide phosphate is the primary and perhaps the only reductant directly used for fatty acid synthesis. The malate- and citrate-cleavage enzymes show marked alterations in activity when overall rates of lipogenesis are altered by dietary or hormonal means; this strongly suggests that the reactions in which these enzymes participate may play a part in controlling the rate of lipogenesis. Consideration was given to the possible role of phosphofructokinase in controlling rates of lipogenesis, and it was concluded that both phosphofructokinase and the citrate-malate cycle may be control points, with an integration of their actions playing key roles.

Gellhorn reported on the modifications of lipid and RNA biosynthesis observed in starvation, aging, and diabetes in rat. During starvation, the incorporation of acetate into the total lipid of adipose tissue and the synthesis of mono-unsaturated fatty acids and RNA are markedly depressed. During aging there is a progressive decline in fatty acid synthesis in adipose tissue. The amount of RNA in adipose tissue from old rats is significantly lower than in young rats, but the specific activity of the RNA isolated from adipose tissue after incubation with labeled uridine is significantly higher in the old. These biosynthetic changes can be reversed by feeding of a fat-free diet to old rats or by injection of insulin. In alloxan diabetes the incorporation of acetate into total lipid decreases, and there is a defect in microsomal monoenoic fatty acid synthesis. Insulin stimulates the renewal of cellular RNA necessary for the synthesis of specific anabolic enzymes. Evidence was obtained which indicates that the informational RNA-ribosome complex formed under the influence of insulin is more stable than the enzyme (or enzymes) synthesized on the template. Gellhorn proposed that the fundamental disturbances in starvation, aging, and diabetes primarily affect RNA biosynthesis and that the enzymatic failures are secondary. The action of insulin in increasing the transport of glucose across cell membranes is not dependent on new enzyme synthesis.

Hormones in Enzyme Biosynthesis

The action of hormones with respect to enzyme biosynthesis was discussed by R. S. Rivlin (Johns Hopkins University) and G. Weber. Rivlin reported that thyroid hormone appears to influence the formation of flavin adenine dinucleotide (FAD), which might in turn regulate the activity of a number of flavoprotein enzymes. He explained the decreased activities of several flavoprotein enzymes in riboflavin or in the case of thyroid-hormone deficiency on the basis of reduced formation of FAD from riboflavin. Because the flavoprotein enzymes are stabilized against degradation by their coenzymes, altered rates of coenzyme synthesis may provide an important mechanism of regulation of enzyme activity in tissue cells.

Weber presented evidence that certain hormones act by influencing whole units of the genome (functional genic units, FGU) governing the biosynthesis of functionally related, key rate-limiting enzymes. The behavior of the key gluconeogenic enzymes under various conditions may be explained by the action of glucocorticoid hormone as an inducer and insulin as a suppressor of the biosynthesis of these enzymes. The similar pattern of behavior he described for the key hepatic glycolytic enzymes (glucokinase, phosphofructokinase, and pyruvate kinase) during development and under conditions of starvation, diabetes, insulin treatment, and steroid administration satisfied the predictions arising from the FGU concept. The following aspects of the integrative action of insulin may be discerned especially as applied to gluconeogenesis and glycolysis in liver: (i) Insulin acts only on one-way, rate-limiting enzymes; (ii) insulin acts on functional units of the genome, resulting in a coordinated increase or decrease in the biosynthesis of functionally related key enzymes; and (iii) insulin achieves its integrative function by a simultaneous but antagonistic action on opposing rate-limiting enzyme systems at the branching points of metabolism.

The special session devoted to the problems of altered enzyme regulation in hepatomas was chaired by S. Weinhouse (Temple University). O. Wieland (University of Munich) discussed the mechanism and possible significance of aerobic formation of acetate in tumor cells. G. E. Boxer (Merck Sharp & Dohme) compared glycolytic enzyme activities in a series of hepatomas from humans and rodents.

Wieland reported that, in four different strains of ascites tumor and in one solid hepatocarcinoma, acetate was an end product of aerobic metabolism of pyruvate and palmitate. According to enzymatic studies with soluble tumor extracts, net formation of acetate can be ascribed to a deficiency of acetate thiokinase as opposed to acetyl coenzyme A deacylase, which is active in tumor cells. The imbalance between enzymatic hydrolysis and rebuilding of acetyl-CoA was offered as an explanation for the observed net production of free acetate by tumor cells.

Boxer reported that the comparison of a series of rat hepatomas of vary-

ing growth rates with a group of human hepatomas showed a similarity with respect to the activities of the enzymes of the glycolytic pathway. In human liver, however, the activities of glucose-6-phosphate dehydrogenase, glycerolphosphate dehydrogenase, and fructose-1,6-diphosphatase are lower than in rat liver, whereas that of phosphoglucomutase is higher in the human tissue. Glucokinase of low glucose affinity was readily demonstrable in the normal liver of the rat, but he found only glucokinase with high glucose affinity in normal liver of the human.

Weber described the "molecular correlation concept" of neoplasia and pointed out that for the design of a rational chemotherapy of neoplastic diseases it is necessary to achieve a critical degree of knowledge of the molecular pattern of cancer cells. There are at least four prerequisites for the elucidation and interpretation of the molecular pattern of the cancer: (i) A suitable model biological system for the biochemical grading of the neoplastic process—this is provided by the spectrum of hepatomas of different growth rates; (ii) identification of key rate-limiting enzymes—modern biochemistry yielded such information; (iii) knowledge of control of metabolic homeostasis—this may be elucidated by study of the regulatory behavior of enzyme and enzyme-forming systems in hepatomas; and (iv) relevance of the metabolic pattern in cancer cells to the neoplastic process—this was approached by correlating the behavior of metabolic pathways and enzymes with the growth rate of liver tumors. He presented detailed evidence for the correlation of alterations in enzyme activities and in behavior of overall pathways in carbohydrate, lipid, RNA, DNA, and protein metabolism; this evidence provides a basis for the molecular correlation concept of neoplasia in hepatoma cells. This pattern is distinctly different from that of the regenerating liver. He concluded that since the progressive alterations in cancer cells reveal a definite pattern at the molecular level, it is now possible to predict the type of metabolic pattern hepatoma cells have on the basis of the growth rate of the tumor.

Drugs: Enzyme Inducers

In the session on enzyme induction by vitamins, hormones, and drugs, R. Kuntzman (Wellcome Research Laboratories) discussed the factors influencing steroid hydroxylases in liver microsomes; C. A. Villee (Harvard Medical School) described the regulation of the biosynthesis of sterols and steroids in the placenta; and R. E. Olson (St. Louis University) outlined the mode of action of vitamin K in inducing the formation of prothrombin.

Kuntzman presented data indicating that those factors which influence drug hydroxylation (for example, age, sex, species, strain, nutrition, hormones, and drugs) also influence steroid hydroxylation by liver microsomes. He suggested that the 16-alpha hydroxylation of testosterone is catalyzed by a different enzyme system than that required for the 6-beta and 7-alpha hydroxylation. Further evidence that separate enzyme systems control the hydroxylation of steroids in various positions came from experiments on developmental and species differences in steroid metabolism.

C. A. Villee presented studies demonstrating the incorporation of acetate and mevalonate into squalene, lanosterol, and cholesterol in minced human placenta, thus providing clues as to the sequence of compounds in the biosynthesis of cholesterol and the site of action of human chorionic gonadotropin in this system. His results form the foundation of further work to determine the nature of regulation of steroid biosynthesis in placenta.

Olson described the results of his investigations undertaken to test the hypothesis that the fat-soluble vitamins act as effector molecules in the model proposed by Jacob and Monod, implying an action at the genetic level. The evidence showed that actinomycin or puromycin inhibited the vitamin-K–induced formation of prothrombin in doses that also blocked synthesis of liver RNA and protein. When vitamin-K–deficient chicks were given vitamin K_3 with and without prior administration of dicumarol, the dose-response curve was suggestive of an allosteric interaction. These data are consistent with the view that the site of antagonism between vitamin K and the coumarin drugs is a molecule which controls DNA-dependent RNA replication.

The session on metabolic regulation through activation, saturation, and feedback was chaired by C. F. Cori (Washington University). C. Peraino (University of Wisconsin) tested the effects of certain hormones on the induction and repression of amino acid-catabolizing enzymes. He suggested that cortisone exerts a "protective" effect on the induction process, permitting induction to occur when carbohydrate is administered, but the steroid itself not causing induction. Similar effects are produced by triamcinolone, but deoxycorticosterone has no effect on the induction or repression phenomena.

Wieland discussed the effects of glucagon and long-chain fatty acids on the production of glucose by isolated, perfused rat liver. He concluded that the production of glucose by the liver may be in part autoregulating. Influx of fatty acids into the liver would be sufficient to stimulate an increase in the formation of glucose. Thus an acceleration of gluconeogenesis would take place whenever fat was mobilized for energy production because of a lack of available carbohydrate.

G. F. Cahill, Jr. (Harvard Medical School), stated that studies on the isolated, perfused rat liver have shown that glucose synthesis and release are primarily a function of the concentration of amino acid and free fatty acid in the medium. Increasing the concentration of free fatty acids augments glucose synthesis from a given concentration of amino acid. These observations and the inability to alter enzyme levels in the perfused liver suggest that the primary determinant of glucose synthesis in such experiments may be the rise in precursor substrate levels.

Adaptation at the Enzyme Level

Mechanisms of adaptation at the enzyme level were reported on by D. M. Gibson (Indiana University) and V. R. Potter (University of Wisconsin). Gibson suggested that the rise in activity of the liver enzymes catalyzing fatty acid synthesis may be attributed to new enzyme formation. Both actinomycin and puromycin block the elevation in enzyme activity which is observed after feeding a high-carbohydrate, fat-free diet to starved rats.

Potter described studies on the enzyme levels in rats adapted to 36-hour fasting. Glycogen, blood sugar, the activities of glucokinase, hexokinase, glucose-6-phosphate dehydrogenase, citrate-cleavage enzyme, tryptophan pyrrolase, tyrosine transaminase, serine deaminase, and ornithine transaminase, and labeling of RNA and DNA in vivo were examined. Several of the enzymes showed great increases in the amplitude of cyclic fluctuations in activity. Certain enzymes showed evidence of a parallel fluctuation, while

others showed individualized timing in peak activity. The "36-hour-fasting adapted" animal may be useful, Potter concluded, in studies on mechanisms of regulating enzyme synthesis or activation and may suggest how human subjects might initiate a program of stress-conditioning.

In the session on behavior of enzymes in vitro, D. B. Villee (Harvard Medical School) discussed the effects of progesterone on enzyme activity of adrenals in organ culture, and J. P. Changeux (Institut Pasteur, Paris, France) discussed allosteric interactions in quaternary structure.

D. B. Villee reported that human fetal and mouse adrenal glands can be maintained for 24 hours in a histologically and enzymatically differentiated state in organ culture. Adrenal glands cultured in the presence of progesterone showed enhanced utilization of progesterone and decreased activity of the 3-β-hydroxysteroid dehydrogenase-isomerase enzyme systems after being homogenized and incubated with progesterone-4-^{14}C and pregnenolone-7-α-^{3}H, respectively. Since these findings may represent examples of control of enzyme activity by substrate and product in mammalian cells in organ culture, such a system is of great experimental interest.

Changeux analyzed the implications of the model proposed by Monod, Wyman, and Changeux which assumes that allosteric proteins able to mediate homotropic interactions are oligomers made up of a small number of identical subunits. These subunits are associated in such a way as to confer at least one axis of rotational symmetry to the molecule. The two most significant implications of this model are (i) that interactions between ligands binding at distinct sites on the same protein molecule may be accounted for even though the binding of one ligand may have no direct effect on the inherent dissociation constant of another, and (ii) that globular proteins involving several identical subunits may possess an element of symmetry and should tend to conserve such symmetry in the event that they undergo a conformational alteration. Various examples of this interesting regulatory process in mammalian enzyme systems were discussed.

Regulation and Isozymes

W. E. Knox (Harvard Medical School) chaired the session on regulation and isozymes. M. K. Schwartz (Sloan-Kettering Institute for Cancer Research) reported on isozymes of aspartate aminotransferase in tissues and blood of man. The values for the Michaelis constant—K_m (L-aspartate)—were the same for the anionic isozyme of heart or liver and were distinctly higher than those for the cationic components. Values for K_m (α-ketoglutarate) were the same for the anionic isozyme of heart or liver and were distinctly lower than those for the cationic components. The relationship of the electrophoretic pattern of aspartate transaminase in human tissues to that in the serum was examined. The appearance of a cationic component in the serum of patients with neoplastic disease was associated with an acute phase of the disease and its disappearance with the subsidence of this phase.

N. Katunuma (Tokushima University, Tokushima, Japan) described the regulation of the urea cycle and tricarboxylic acid cycle by ammonia. A new metabolic pathway of reduced nicotinamide adenine dinucleotide (NAD) and reduced NAD phosphate to nicotinamide, including reduced pyridine nucleotide pyrophosphatase and nicotinamide mononucleotide oxidase, was first proved to occur in mitochondria of rat liver. The new pathway was accelerated by addition of ammonia. High-protein diet, administration of cortisone, or diabetes markedly increased the hepatic supernatant aspartate and alanine transaminase isozymes, but did not affect the mitochondrial transaminase isozymes.

The special symposium lecture was given again this year by Sir Hans Krebs (Oxford University). Krebs discussed the regulation of the release of ketone bodies by the liver, bringing evidence to reconcile the previously contradictory findings. He distinguished between the mild ketosis of starvation of low-carbohydrate diets, which is a useful process, and the severe ketosis of the diabetic coma and the lactating cow, which is uncontrolled ketone-body formation and is harmful. By the recognition of the association of severe ketosis with excessive rates of gluconeogenesis which, in turn, drains off oxaloacetate into gluconeogenesis, an advance was made in the understanding of the mechanisms involved. The gluconeogenic drain results in a decline of oxaloacetate level which, in turn, decreases the energy supply through the tricarboxylic acid cycle when the concentration of oxaloacetate decreases below the K_m value of the condensing enzyme. In consequence, the liver must perform an excessive oxidation of fatty acids to acetyl-CoA, which is then formed in excess of the capacity of the condensing enzyme and therefore results in ketone-body formation. Krebs concluded that the abnormal formation of ketone bodies is a type of respiration forced upon the liver when excessive gluconeogenesis, namely, excessive conversion of oxaloacetate to phosphoenolpyruvate, limits the rate of the tricarboxylic acid cycle. Since the severe ketosis arises from high rates of gluconeogenesis, clinical treatment must aim at a reduction of the need for gluconeogenesis. In diabetics the administration of insulin or glucose plus insulin will achieve this result. In bovine ketosis this aim is most readily obtained by the parenteral administration of appropriately large quantities of glucose.

Krebs's lecture was a beautiful example of problem-solving at the highest level of penetrating biochemical analysis in both the molecular and the clinical spheres. Thus it was a fitting and satisfying conclusion to a stimulating and useful meeting.

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GEORGE WEBER
Pharmacology Department,
Indiana University School of Medicine,
Indianapolis

Catecholamine Symposium

The first Catecholamine Symposium which was held in 1958 played an important role in stimulating the many advances which were subsequently made in this field. The developments since that first symposium have been so many and of such importance that a second symposium was organized and held at the Istituto Di Ricerche Farmacologiche Mario Negri in Milan, Italy, 4–9 July 1965.

U. S. von Euler (Sweden) gave the opening address entitled "Twenty years of noradrenaline," commemorating his