# Meetings

## Enzyme Regulation in Mammalian Tissues

The regulation of enzyme activity and synthesis in mammalian tissues was the theme of the third international symposium held 5 and 6 October 1964 at the 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 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 this function of regulation of enzymes and metabolic pathways in hepatic cancer.

Regarding the mechanism of the effect of adrenocorticosteroid hormones on enzyme-forming systems, F. Kenney (Oak Ridge National Laboratory) and M. Feigelson (Columbia University) discussed the action of these hormones on RNA synthesis in different organs. H. L. Segal (State University of New York at Buffalo) and G. Weber (Indiana University) discussed the effects of these hormones on the biosynthesis of liver enzymes. Kenney pointed out that the rapid increase in rate of nuclear RNA synthesis precedes accumulation of the induced tyrosine transaminase in a fashion consistent with an interpretation that the primary hormonal effect was stimulation of synthesis of RNA, which in turn resulted in an increased rate of synthesis of specific enzyme protein. Feigelson showed that stimulation of hepatic purine biosynthesis occurred as a marked and early response to glucocorticoids. Further work revealed that, when administered intraperitoneally to adrenalectomized rats, L-glutamate, L-alanine, L-leucine, their D-counterparts, inorganic ammonium salts, and L-glutamine all mimic cortisone in the stimulation of glycine-$^{14}$C incorporation into acid-soluble adenine. Cortisone, in liver, stimulated incorporation of glycine into protein and acid-soluble purine but evoked a marked depression in these metabolic pathways in lymphoid tissues, thymus, and spleen.

Segal measured the half-life of rat liver glutamic-alanine transaminase and the relative rates of incorporation of labeled amino acid in vivo and demonstrated that the administration of glucocorticoid hormones resulted in an increased rate of synthesis of the enzyme. He emphasized the importance of studies on rates and conditions of enzyme degradation in control of tissue enzyme levels.

Weber suggested on the basis of metabolic flow and regulatory significance that the enzymes governing the steps from glucose to lactate and the reverse are divided into three groups. Key gluconeogenic and glycolytic enzymes exhibit low activity and govern one-way reactions, and thermodynamic considerations and organ distribution may be relevant. In contrast, bifunctional enzymes have high activity and catalyze reversible reactions; no thermodynamic barriers are involved, and these enzymes are present in all organs. The four key enzymes of gluconeogenesis may occupy one genetic unit, since they are gradually decreased in hepatomas and are completely absent in rapidly growing liver tumors. Weber presented evidence that both exogenous and endogenous adrenocortical hormones act as inducers of the liver gluconeogenic enzymes. He correlated RNA specific activity, amino acid levels, and the sequence of induction of these enzymes in liver. Additional experiments were cited in support of the suggestion that insulin is capable of acting as a suppressor of gluconeogenic enzymes. When rats are made diabetic by means of alloxan, enzyme activities increase; this increment can be blocked by insulin and also by actinomycin, puromycin, or ethionine. The increased enzyme activities in the diabetic rat can be brought back to normal by insulin. On withdrawal of insulin, enzyme activities rapidly rise. Insulin was found to be capable of blocking the triamcinolone-induced primary synthesis of hepatic gluconeogenic enzymes and was also able to inhibit the already established synthetic process after several days of hormone treatment and to return enzyme activities to normal. In diabetic rats a marked rise in incorporation of labeled orotate into RNA was found; insulin returned the elevated specific activity to normal in one day.

### Muscle Enzymes

The regulatory properties and functions of muscle enzymes were explored by S. Weinhouse (Temple University) and E. Helmreich and C. F. Cori (Washington University).

Weinhouse focused attention on the possibility that the splitting of fructose to trioses might be a rate-limiting step for glucogenesis from fructose, and he reported extensive data on the properties of aldolase. He provided evidence that liver aldolase differs from muscle aldolase at the binding site of the substrate to the enzyme. He reported that muscle aldolase was competitively inhibited by adenosine triphosphate (ATP) but only to a lesser degree by adenosine monophosphate (AMP). In contrast, hepatic aldolase was competitively inhibited by AMP to a much greater extent than by adenosine diphosphate (ADP), but was not inhibited by ATP. The lack of inhibition of liver aldolase by ATP suggested that the hepatic enzyme differs from the muscle enzyme in that it lacks a binding site for the 6-phosphate of fructose-1,6-diphosphate. Evidence was also found which suggests that the liver enzyme differs further from the muscle enzyme in having a binding site for the carbon-6-methylane group of fructose-1-phosphate or fructose-1,6-diphosphate. The marked differences between hepatic and muscle aldolases in their responses to ATP may be related to differences in function. In muscle where the primary function is contraction, glycolysis in excess of the need for ATP is wasteful. In contrast, liver utilizes the ATP produced for a number of biosynthetic processes with release of AMP. The inhibition of liver aldolase by AMP

may provide a feedback mechanism by a product for control of the level of ATP.

E. Helmreich and C. F. Cori (Washington University) reported results bearing on the connection between mechanical and chemical processes in skeletal muscle. Two reactions were of prime importance in the regulation of glycolysis in frog skeletal muscle: the formation of glucose-1-phosphate from glycogen and inorganic phosphate through action of the phosphorylase system, and the removal of fructose-6-phosphate through the phosphofructokinase reaction. They concluded that during electrical stimulation of the muscle these two enzyme systems increased their activity synchronously and proportionally to the number of stimuli. After stimulation the enzyme activities returned rapidly to the resting rate. In the investigation of these mechanisms the authors also utilized an interesting strain of mice incapable of forming phosphorylase *a* during stimulation. It was suggested that the activation and inactivation of at least two of the glycolytic enzymes are geared to the contractile process itself.

The behavior of enzyme-forming systems in liver slices and in perfused liver was examined by R. C. Haynes, Jr. (Western Reserve University), A. B. Eisenstein (Washington University), R. L. McGeachin (University of Louisville), G. F. Cahill, Jr. (Harvard Medical School), and A. B. Hastings (Scripps Clinic and Research Foundation). Haynes showed that his liver-slice system responded to the glucocorticoid, triamcinolone, with an increase in gluconeogenesis; he was able to exclude transmembrane movement of L-alanine as playing a role in this effect. The transamination of alanine also did not occur. He suggested that triamcinolone primarily affected reactions occurring in the mitochondrion by way of the pyruvic carboxylase reaction.

Eisenstein found that glycogen deposition and a rise in glutamic pyruvic transaminase as a response to glucocorticoid stimulation were markedly less in pyridoxine-deficient rats than in normal ones. However, the response to adrenal steroids was equally good in pyridoxine-deficient and in normal liver slices. Puromycin aminonucleoside, an inhibitor of protein synthesis, abolished the increase in gluconeogenesis induced by adrenocortical hormones.

McGeachin demonstrated that amylase synthesis in perfused normal rat liver was blocked by puromycin, dinitrophenol, anoxia during perfusion, or previous damage to the liver. He estimated the half-life of hepatic amylase at about 4 hours.

Cahill reported that in the isolated, perfused rat liver glucagon caused the appearance of new hexoses in the perfusate. He presented evidence that a significant effect was obtained within 20 minutes after addition of glucagon to the perfusate. Since a number of gluconeogenic precursors resulted in increased production of hexose by glucagon, Cahill suggested that the gluconeogenic sequence worked as a single unit; this accords with the suggestions of Weber and, for glycolysis, with the results of Helmreich and Cori.

Hastings reported on the role of carbon dioxide and *p*H as regulatory factors in metabolism. His previous work showed that increasing the *p*H from 7.0 to 7.7 markedly increased glycogen synthesized in liver slices. He now reported that increasing the concentration of $CO_2$ from 10 m*M* to 45 m*M* at constant *p*H caused an equally large increase in glycogen synthesized. High $CO_2$ greatly enhanced adenosine triphosphatase activity; however, hexokinase, glucokinase, glycerokinase, phosphorylase, glycogen synthetase, and pyrophosphatase were not affected. When *p*H was raised from 7.1 to 7.6 at constant $CO_2$ tension the ratio of acetate to $CO_2$ was decreased 62 percent, but the ratio of acetate to fatty acid was increased 300 to 400 percent. When $CO_2$ concentration was raised from 10 m*M* to 40 m*M* at constant *p*H a 4- to 6-fold increase of conversion of acetate to fatty acids was observed. The conversion of acetate to fatty acids was much more pronounced in the high potassium medium than in the high sodium medium. Hastings reported 20 years ago that the high-potassium medium was required for the conversion of glucose to glycogen by liver slices, and now from the present experiments it is clear that potassium ions are equally essential for the conversion of acetate to fatty acids.

Regulation of enzymes during development was discussed by D. G. Walker (University of Birmingham, England), H. N. Christensen (University of Michigan), H. B. Burch (Washington University), and C. A. Villee (Harvard Medical School). The control mechanisms of carbohydrate metabolism in developing mammals and the failure of certain hepatic control mechanisms prior to maturation were reviewed by Walker. He reported that glucokinase develops immediately after birth in guinea pigs, but only after 16 days in rats. The development of glucokinase is due not to an activation process but to *de novo* synthesis. The absence of glucokinase from rat liver prior to the 16th day was not due to lack of insulin, although this hormone was capable of inducing glucokinase in the adult. The course of glucokinase development was not affected by infusion of glucose, chlorpropamide or exogenous insulin, or insulin plus glucose. Glucokinase synthesis was impaired when rats were starved during the neonatal period. However, when the starved rats were fed they were capable of synthesizing glucokinase, and this was prevented by ethionine, *p*-fluorophenylalanine or actinomycin D.

Burch pointed out that differentiation, growth, and development have been far better described morphologically than biochemically, although chemical factors presumably bring about the differentiation. She reported studies on biochemical differentiation in rat liver as studied by measurement of enzyme activities, substrate levels, and product concentrations in quick-frozen tissues. During development in the liver there was a decrease in capacity for glucose utilization and glycolysis and an increase in capacity for glycogenolysis, glucose formation, and lactate utilization.

Christensen related the changes in amino acid transport activity with developmental alterations. During the first day of extrauterine life of the guinea pig, the liver greatly intensified the extent of the steady-state concentration of two amino acids, one normal (glycine) and one non-metabolizable (1-aminocyclopentanecarboxylic acid).

Weber pointed out the value of using the guinea pig rather than the rat in developmental studies; in the rat the liver is largely a hemopoetic organ in the embryonic and in early postnatal state, and consequently metabolic and enzyme alterations in the livers of embryonic, and even newborn, rats may represent a change in cellular population rather than metabolic transformation.

Villee discussed biochemical and genetic aspects of differentiation. He pointed out that differentiation could conceivably occur at the level of genic

DNA, of messenger RNA, of protein synthetic process occurring on ribosomes, or of the ultimate protein product, enzyme. Since specific enzymes appear sequentially in the course of development, this should mean that the specific DNA that provided the code for a given protein was activated and the burst of messenger RNA was released. Villee presented data referring to the five malic dehydrogenases and their development in sea urchins. Embryos developing in sea water containing D- or L-malate show that under certain circumstances those grown in D-malate had one additional band of L-malate dehydrogenase.

The blocking effects of actinomycin and puromycin on induced enzyme synthesis were discussed in a number of papers. The newer and more unusual effects of these antibiotics were discussed by F. Moog (Washington University), J. Ashmore (Indiana University) and C. A. Nichol (Roswell Park Memorial Institute). Moog reported that the administration of puromycin or actinomycin accelerated the physiological rise of alkaline phosphatase activity of mouse duodenum. Both antibiotics also resulted in a precocious increase of phosphatase activity; if hydrocortisone was given at the same time, the effects of hormone and antibiotic were additive. Colchicine achieved results similar to those found with actinomycin. Ethionine also increased phosphatase activity, but only in high concentrations. The specificity of these responses is signalled by the fact that under the same experimental conditions the phosphatase in jejunum or in kidney was not affected or decreased.

Ashmore studied the effect of these antibiotics in a liver-slice system where, in the absence of these compounds, triamcinolone stimulated incorporation of alanine and $CO_2$ into glucose and protein. Addition of actinomycin to the in vitro system reduced the response to triamcinolone. Puromycin caused a marked reduction in incorporation of alanine into protein and inhibited the effect of triamcinolone on incorporation of alanine into glucose. Actinomycin or puromycin did not block the effect of triamcinolone on incorporation of $CO_2$ into glucose. In the presence of both triamcinolone and puromycin, incorporation of alanine into protein was inhibited by 85 to 90 percent. Thus both steroid and puromycin inhibited protein synthesis in liver slices, and their effects were additive.

Nichol reported that actinomycin D increased the activities of alanine transaminase and serine dehydrase in normal or in adrenalectomized rats in 3 days.

W. E. Knox (Harvard Medical School) and O. Greengard (Institute for Muscle Disease) presented a comprehensive review on regulation of enzymes of nitrogen metabolism. Particular emphasis was placed on the nutritional and hormonal regulatory influences on tryptophan pyrrolase, transaminases, xanthine oxidase, arginase, and glutaminase.

### Enzyme Regulation in Hepatomas

As in previous years a special session was devoted to the unique problems of altered enzyme regulation in hepatomas of different growth rate. Weinhouse introduced the subject and described the behavior of glucose-ATP phosphotransferases during azo-dye hepatocarcinogenesis in the rat. The results indicated that, along with extensive early bile-duct proliferation, there was a reversal in phosphotransferase pattern; hexokinase increased with bile-duct proliferation, whereas glucokinase decreased as the parenchymal cell population decreased. He suggested that the hepatocellular carcinomas carry in subsequent generations the genetic apparatus for hexokinase formation because all tumors had hexokinase activity, although to different degrees. In most of the Morris hepatomas of different growth rate the glucokinase was lost; however, it was present in some. It is of significance that it was present in normal activity in an early generation of the most highly differentiated hepatocarcinoma studied so far, the 7787. H. P. Morris (National Cancer Institute) reported that glutamic-oxalacetic transaminase appeared in two isozymes in hepatomas 5123-A, 5123-B, 7316-B (slow growing), 7288-C (medium growth rate), and 3683 (rapidly growing). The electrophoretic mobilities of the isozymes were similar to those of control preparations of normal liver. The relative activities of the two isozyme peaks were different in the different hepatomas.

J. S. Roth (University of Connecticut) investigated the behavior of aspartate aminotransferase activity because the availability of aspartate for RNA synthesis might be an important regulatory factor determining the rate of cell proliferation. Administration of cortisone doubled the homogenate enzyme activity in 6 days, whereas thyroxine, growth hormone, estradiol, or testosterone was ineffective. Six daily injections of L-aspartate led to a modest increase in the enzyme activity which was abolished by actinomycin or puromycin. Induction of enzyme activity by cortisone was not blocked by actinomycin, but puromycin prevented the induction to the extent of about 70 to 80 percent. Neither cortisone nor aspartate induced aspartate aminotransferase activity in the 5123-D, Dunning or Novikoff hepatomas or in embryonic rat liver. In contrast, regenerating liver gave the same response as normal liver.

H. C. Pitot (University of Wisconsin) employed a forced-feeding regimen and found that the template stability of threonine dehydrase, ornithine transaminase, and tryptophan pyrrolase in hepatomas is different, and usually less, than that found in liver. In contrast, the thymidine kinase template was more stable in the hepatomas. Pitot suggested that the defective control of enzyme synthesis in hepatomas may be related to alterations in the stability of RNA templates in tumor cells and that such aberrations underlie the molecular basis for the abnormal growth observed in hepatomas.

Weber described the correlation of metabolic phenotype with growth rates in the Morris hepatoma spectrum. He found that in slow-growing tumors the gluconeogenic enzymes may be induced to a minor extent compared to normal liver. However, the enzymes are absent and fail to respond to hormonal stimulation in the rapidly growing hepatomas. Corresponding to the absent enzymatic response there was also a failure to respond with increased incorporation of labeled orotate or uracil into total RNA and with the rise in amino acid level which characterize the hormone response in normal liver. Weber pointed out, however, that a similar response of the enzyme forming systems of the gluconeogenic enzymes occurs in kidney cortex. Thus, a lack of response to hormonal stimulation is not unique to liver tumors.

T. Hultin (Wenner-Gren Institute, Stockholm, Sweden) discussed the action of carcinogenic amines in terms of their effects on hepatic protein metabolism which in short-term experiments can be separated in time. With-

in the first few hours there was an inhibition of ribosomal protein synthesis, and experiments with artificial messenger polynucleotides suggested that there was a decreased stability of attached messenger RNA in addition to a direct damage to the ribosomes. A few hours later the incorporation markedly rose often far beyond the normal level. The stimulatory effect was mainly due to an increased sensitivity of hepatic cells to the systemic level of corticosteroids. Hultin suggested that chemical liver carcinogenesis was characterized by a pronounced adrenal dependence.

Some of the newer concepts in enzyme regulation were discussed by S. Numa (Munich, Germany), B. C. Goodwin (University of Edinburgh), and V. R. Potter (University of Wisconsin). Numa emphasized that the carboxylation of acetyl-CoA to form malonyl-CoA is the first step and the rate-limiting step in the biosynthetic sequence in the overall conversion of acetyl-CoA to fatty acids. He reported that acetyl-CoA carboxylase was strongly inhibited by addition in vitro of long-chain acyl-CoA derivatives and that prolonged fasting or diabetes decreased the enzymatic activities. It was found that this biotin-containing enzyme was activated by citrate or other tri- and dicarboxylic acids. He suggested that conformational changes of the enzyme protein may play a role in the regulation of its activity.

Goodwin emphasized that the essential control processes of cells involve continuing oscillatory activity, so that the cell is constantly cycling through a set of states with periods determined by rates of fundamental biochemical activities, such as macromolecular synthesis and enzyme-catalyzed metabolic transformations. Detailed dynamic analysis of feedback repression and the equations describing the dynamics of such processes defined nonlinear biochemical oscillators. Goodwin developed the viewpoint that the dynamics of enzyme regulation in cells is intrinsically oscillatory and that this oscillatory activity represents a type of biological energy.

V. R. Potter discussed the implication of these concepts and presented enzymatic evidence that cellular processes are intrinsically rhythmic. However, the enzyme behavior in hepatomas fails to respond to the biological timing signals.

The symposium was sponsored by Damon Runyon Memorial Fund, Inc., Indiana University School of Medicine, American Cancer Society Institutional Grant, the Burroughs Wellcome Co., Merck Sharp & Dohme, and the Upjohn Co. The full text of the papers, edited by George Weber, will be published in the spring of 1965 as volume 3 of *Advances in Enzyme Regulation* (Macmillan, New York; Pergamon Press, Oxford). Volumes 1 and 2 of this series of conferences on enzyme regulation in mammalian tissues were published in 1963 and 1964 and presented the proceedings of the first two symposia.

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## Diffusion in Oceans and Fresh Waters

Of the 20 papers presented at the symposium on diffusion in oceans and fresh waters sponsored by and held at the Lamont Geological Observatory, Palisades, New York, from 31 August to 2 September, 12 were directly concerned with diffusion of dyes. The remaining papers covered topics from sodium vapor trails in the upper atmosphere to the diffusion of radon-222 near the sea bottom.

C. G. Gunnerson (U.S. Public Health Service) discussed the physical and chemical properties of rhodamine-B and Pontacyl Pink-B dyes and gave loss rates due to oxidation, photochemical decay, and absorption. A significant result was that, in the presence of suspended solids, as much as 85 percent of these dyes can be lost by absorption, thus necessitating *in situ* field calibration for loss rates. Greatly appreciated was the recounting by D. W. Pritchard (Johns Hopkins University) of practical lessons learned from years of field experience at Chesapeake Bay Institute. These ranged from cautions against sampling rhodamine-B through rubber, Tygon, or copper tubing to description of techniques which increase the sensitivity of fluorometers, thereby permitting the use of less dye, reducing the cost of the tests, and producing "cleaner," more easily interpretable data.

Three papers dealt with pollution dispersion in rivers and estuaries. J. F. Wilson, Jr., and W. E. Forest (U.S. Geological Survey) presented results of time-of-travel studies in the Potomac River; C. C. Kisiel (University of Pittsburgh) gave results of dye experiments in the Ohio River; and D. O'Connor (Manhattan College) gave results of studies on pollutant dispersion in estuaries. O'Connor commented on the magnitude of the pollution problem relative to the present crude level of theory; in New York City alone, the cost of needed new sewage plants is about $100 million. One general result of sewage dispersion studies is that outfall design is not strongly affected by order of magnitude variations in eddy diffusion coefficients, and that advective circulation is a significant factor about which more knowledge is needed.

Six papers were presented concerning dye diffusion studies in coastal and oceanic waters. R. Reinert (New York University) discussed surface diffusion from a continuous point source in coastal waters. A phenomenon observed by Reinert (as well as by other workers) was the tendency of dye to form elongated bands and streaks in the direction of the wind and waves, with dye being closer to the surface and more concentrated on the leeward side of the patch and deeper on the windward side. The width of the dye plume as a function of downstream distance exhibited neither linear nor square-root dependence as predicted by theory. T. Ichiye (Lamont Geological Observatory) gave results of diffusion experiments in which dye techniques were used in coastal waters; offshore observation of dye patches again yielded elongated, irregular patterns with banding and heavy striations. The patches also showed consistent large curvatures which Ichiye related to Ekman spiral formation; similar observations in the southern hemisphere gave reverse curvatures. In discussion, Pritchard presented data from the Cape Kennedy region showing similar Ekman spiral effects. If these effects are indeed attributable to Ekman spiral flow, then we have the unusual situation of discovery of a long sought-after classical and analytical phenomenon by means of experiments designed to give information about turbulent, random phenomena.

J. E. Foxworthy (University of Southern California) reported on dye diffusion experiments to determine eddy diffusion in California coastal waters, using a Los Angeles offshore out-