Meetings

Enzyme Regulation in Mammalian Tissues

The Eighth International Symposium (*1*) on Regulation of Enzyme Activity and Synthesis was held at Indiana University School of Medicine on 29 and 30 September 1969. In the special symposium lecture H. A. Krebs (Radcliffe Infirmary, Oxford, England), in describing the rate control of the tricarboxylic acid cycle (Krebs cycle), reported that, in rat liver homogenates, the rates of ketone body formation due to addition of long-chain fatty acids are increased by the addition of adenosine triphosphate (ATP), cytochrome c, carnitine, and α-oxoglutarate. Such substances direct acetyl coenzyme A (CoA) formed from oleate to produce ketone bodies instead of entering the Krebs cycle. The cycle is inhibited, and a greater proportion of the oxygen uptake is due to the oxidation of fatty acids to acyl-CoA. The concentration of oxaloacetate does not limit the entry of acetyl CoA into the cycle. Krebs's findings were consistent with the assumption that an inhibition of citrate synthase by ATP is an important factor regulating the fate of acetyl CoA in the homogenates. Uncoupling agents that decrease the concentration of ATP cause an increase in activity of citrate synthase (E.C. 4.1.3.7) and are thus "antiketogenic." The ATP is not the only factor controlling the activity of citrate synthase. The concentration of oxaloacetate can also be rate-limiting in situations where the redox state of the nicotinamide-adenine dinucleotide couple in the liver cell changes in the direction of reduction. At a given concentration of malate this causes a decrease in the concentration of oxaloacetate.

In a session on Control Mechanisms in Carbohydrate Metabolism, G. F. Cahill, Jr. (Harvard Medical School, Boston) discussed the control mechanisms of gluconeogenesis in fasting man. Glucagon rapidly reduces circulating levels of amino acids and accelerates production of urea until a net steady state is approached with reduced levels of amino acid and a return to normal rates of gluconeogenesis, as evidenced by nitrogen excretion. These data suggest that glucagon may accelerate the gluconeogenic potential of liver for a given concentration of substrate, but that alanine concentration, as controlled by its peripheral formation and release from muscle, plays the dominant role. G. Weber (Indiana University School of Medicine, Indianapolis) described the inhibitory effect of phenylalanine and phenylpyruvate on pyruvate kinase and hexokinase in the human brain and its possible relevance to brain damage in phenylketonuria. Activity of pyruvate kinase from brain of human adults was inhibited by L-phenylalanine, yielding a K_i of 8.5 mmole/liter. Activities in samples of 13-, 14.5- and 21-week-old human fetuses were inhibited by L-phenylalanine concentrations of the same magnitude. The activity of the pyruvate kinase and hexokinase from brains of human fetuses was less than 10 percent of that in the adult. The low activity of these enzymes in fetal brain could render them more vulnerable to inhibition by L-phenylalanine. The inhibition of these glycolytic enzymes appears to be selective, since a number of other enzymes of carbohydrate metabolism were not inhibited. The inhibition of activity of pyruvate kinase from brain by L-phenylalanine may cause a decrease in the rate of production and concentration of nucleoside triphosphates which are required in synthesis of lipids, proteins, DNA, and RNA. Since brain development entails a gradual increase in the ATP, GTP, and UTP, an inhibition of pyruvate kinase might lead to a serious interference with biosynthetic cellular functions as well as cellular multiplication. This would explain, at least in part, the mechanism of brain damage in individuals with phenylketonuria. T. E. Mansour (Stanford University School of Medicine, Stanford) described the kinetics and physical properties of phosphofructokinase and its role in controlling glycolysis. In addition to control by allosteric interaction, the results from studies on phosphofructokinase in flukes suggest regulation through an association-dissociation mechanism.

In a session on Metabolic and Enzyme Control in Avian Systems, F. Maley (New York State Department of Health, Albany) reported that the regulation of deoxycytidylate deaminase in chick embryos is markedly allosteric. The enzyme is differentially sensitive to sulfhydryl reagents, ethylenediaminetetraacetic acid, urea, sodium dodecylsulfate, and proteolytic digestion in the presence of the effectors. In all of these cases deoxycytidine-5′-triphosphate protects the deaminase against denaturation, whereas deoxythymidine-5′-triphosphate enhances the enzyme's susceptibility to denaturation. N. Katunuma (Tokushima University School of Medicine, Tokushima, Japan) pointed out differences in glutamine metabolism of mammals and birds. Glutaminase activity in birds was less than one-tenth of that in mammals, and strong inhibition of product formation by glutamate was observed. Liver glutaminase is inducible in mammals by high protein diet but not in birds. Glutamine synthetase and glutamine-phosphoribosylpyrophosphate-amidotransferase are induced in birds by high protein diet but not in mammals.

In a session on Control Mechanisms in Microorganisms, H. Holzer (Freiburg University, Freiburg, Germany) discussed some aspects of the regulation of metabolism by the adenylic acid system and presented evidence for an enzyme-catalyzed chemical modification of phosphofructokinase from yeast by partners of the adenylic acid system. E. R. Stadtman (National Institutes of Health, Bethesda, Maryland) described the subunit structure of glutamine synthetase [E.C. 6.3.1.2] as disclosed by physical measurements and by electron microscopy. There could be 384 multiple molecular forms of glutamine synthetase, if a random attachment of adenylyl groups to the 12 subunits occurs. Interactions between adenylylated and unadenylylated subunits within the same molecule may influence the affinity of the subunits for substrates but may not significantly affect the maximum catalytic potential, especially with respect to γ-glutamyltransferase activity. Nonenzymatic nitration of up to two tyrosine residues per subunit of unadenylylated enzyme produces effects that are similar to that obtained by enzyme-catalyzed adenylylation.

R. W. Estabrook (The University of Texas Southwestern Medical School, Dallas), in a session on Regulation in Different Organs, presented spectro-

photometric studies of microsomal and mitochondrial cytochrome oxidation and reduction in liver and adrenal slices. The potential for cytoplasmic reduced pyridine nucleotides governing the diversion of reducing equivalents to the microsomal electron-transport chain has been evaluated by fluorometrically measuring their state in similar slices. J. Himms-Hagen (University of Ottawa, Ottawa, Canada) reviewed the regulation of metabolic processes in brown adipose tissue in relation to nonshivering thermogenesis. To obtain an estimate of the contribution of the brown adipose tissue to total nonshivering thermogenesis in rats acclimated to cold, the tissue was removed and the calorigenic response of the rats to intravenous infusions of noradrenaline was measured at intervals thereafter. Since there is only a progressive loss of response over 3 to 4 days, she suggested that brown adipose tissue may have a second important function in nonshivering thermogenesis in addition to the production of heat, namely, that of an endocrine gland whose secretion modifies the capacity of the other tissues for nonshivering themogenesis. A. C. Sartorelli (Yale University School of Medicine, New Haven) studied the effect of partial hepatectomy on the induction of drug-metabolizing enzymes in order to investigate the ability of liver cells to respond to the competitive demands of growth and the functional requirement for drug metabolism. The results indicate (i) that the requirement for growth predominates over functional response, (ii) that the operation of a process sensitive to x-irradiation that controls the preferential expression of cellular proliferation or functional response to inducer occurs about 1 hour after partial hepatectomy, and (iii) that conditions of protein depletion prevent both cellular proliferation after partial hepatectomy and the delay of the induction of hepatic drug-metabolizing enzymes caused by the operation.

In a session on Regulation of Enzymes in Glycogen Metabolism, H.-G. Hers (University of Louvain, Louvain, Belgium) discussed the regulation of glycogen synthesis in the liver. He proposed that there is a continuous interconversion of the two forms of glycogen synthetase in vivo; the inactivating system normally predominates. The administration of glucose (and possibly of glucocorticoids) stimulates the synthetase phosphatase, bringing its activity above that of the kinase. This glucose effect is controlled by the level of glycogen. The inactivating effect of glucagon is explained by the action of cyclic adenylic acid on the kinase.

E. G. Krebs (University of California School of Medicine, Davis) said that the finding that the receptor for cyclic AMP in the phosphorylase activation system is a protein kinase suggested that other metabolic effects of this regulatory nucleotide may be mediated by protein phosphorylation reactions. The nature of the substrates for these reactions would depend upon the particular metabolic effect in question. One cyclic AMP–dependent protein kinase might catalyze all of the hypothetical phosphorylation reactions, or there could be a specific kinase for each reaction. In an attempt to answer these questions, the protein kinases of a number of mammalian tissues were investigated. From three of these, skeletal muscle, heart muscle, and adipose tissue, the cyclic AMP–dependent kinases were purified and characterized, and the substrate specificities were studied. Each of these protein kinases is inhibited by a heat-stabile protein inhibitor obtained from skeletal muscle. S. Mayer (University of California at San Diego, La Jolla) described the regulation of phosphorylase-activating pathway in intact cardiac and skeletal muscle. In the heart there are at least two receptor sites for activation of adenyl cyclase—one for epinephrine which is blocked by beta adrenergic blocking agents and one sensitive to glucagon. The catalytic activity of phosphorylase kinase, as opposed to the cyclic AMP–dependent activation of the enzyme, requires Ca^{2+}. When Ca^{2+} was removed from the medium during perfusion of rat hearts, epinephrine caused formation of cyclic AMP but not transformation of phosphorylase *b* to phosphorylase *a*. Excess Ca^{2+} produced conversion of *b* to *a* in the absence of epinephrine. Very small doses of this amine produced activation of kinase in intact dog hearts without conversion of *b* to *a*. Regulation of phosphorylase activation by calcium is further suggested by experiments in which electrical stimulation of skeletal muscle produced rapid formation of phosphorylase *a* without either formation of cyclic AMP or kinase activation. Thus the release of intracellular Ca^{2+} by hormone may provide a second mechanism of regulation of the activation of the phosphorylase system. It may be the primary mechanism in the case of activation associated with excitation and contraction of skeletal muscle.

In a session on Regulation through Hormone Action, O. B. Crofford (Vanderbilt University School of Medicine, Nashville) postulated that the binding of insulin to the receptor induces a conformational change within the receptor which then generates a signal to the cell. Among other effects, it activates the glucose transport system and reduces the concentration of cyclic AMP within the cell. R. S. Rivlin (College of Physicians and Surgeons, Columbia University, New York) demonstrated that activities of flavoprotein enzymes are decreased in both riboflavin deficiency and hypothyroidism and that thyroid induction of at least one flavoprotein enzyme is markedly reduced in riboflavin deficiency. His results provided further evidence that the rate of coenzyme synthesis may govern the accumulation of flavoprotein enzymes in both riboflavin deficiency and hypothyroidism.

In a session on Enzyme Induction in vitro, F. Sereni (Milan University Medical School, Milan, Italy) studied the factors controlling activity of tyrosine aminotransferase during fetal life in order to determine the mechanisms by which spontaneous induction occurs in vitro. Hydrocortisone showed an additive effect, whereas glucagon was effective in inducing activity of tyrosine aminotransferase only when spontaneous development had not already taken place. Actinomycin D prevented in part the spontaneous development of the enzyme. It seems probable that the tyrosine aminotransferase development in vitro occurs by a mechanism similar to the one involved when glucagon induced the increase of the same enzyme activity in vivo. J. C. Houck (George Washington University School of Medicine, Washington, D.C.) reported that as much as 30 percent of the collagen of the skin of rats was lost within 24 hours after injection of cortisol. Within 6 hours after steroid injection, activity of a free collagenolytic enzyme could be determined extracellularly. This activity was almost completely eliminated by prior treatment of these animals by either cycloheximide or actinomycin D.

In a session on Regulation and Isozymes, N. Katunuma described the noninherent distribution of glutaminase isozymes in hepatomas of different growth rates. He did not observe any direct relation between the growth rate

in cancer cells and the degree of differentiation of this organ-specific isozyme whose pattern changes irreversibly in the course of development. The activity of the marker enzyme does not change in regenerating liver or after various hormonal treatments. H. Inoue (University of Wisconsin Medical School, Madison) observed two forms of the enzyme serine dehydratase in liver and hepatomas. The two forms appear to be regulated by different environmental mechanisms. Serine dehydratase in the rat occurs only in the liver. These studies again emphasize that the mammalian cell must have considerably greater flexibility in the regulation of enzyme synthesis than the bacterial cell. This may in part be due to the complex structure of the mammalian cell with spatial separation of the synthesis of different forms or to even more subtle distinctions in the regulation of the synthesis of such closely related isozymes.

In a session on Control Mechanisms in Tumors, V. R. Potter (University of Wisconsin Medical School, Madison) described the induction of an enzyme in Morris hepatoma 9618-A that was thought to be noninducible. Tyrosine transaminase in hepatoma 9618-A is very low in activity on standard diets and is unaffected by a 60 percent protein diet or by hydrocortisone injections, either of which induces high amounts of activity in normal liver. Thus it might have been assumed to be "uninducible" or "deleted." Potter reported that such interpretations were now untenable and that the lack of an enzyme or the failure to induce an enzyme under conditions that result in enzyme induction in differentiated tissues of adults no longer suffices to define the state of the genome in a neoplasm. The experiment in which the enzyme was induced in the hepatoma was the culmination of numerous trials using inductive procedures designed on the assumption that the hepatoma cells resemble fetal cells more closely than they resemble adult liver cells. G. Galli (University of Milan, Milan, Italy) investigated the latest stages of cholesterol biosynthesis in rat liver, in growing and adult central nervous systems, and in experimental and spontaneous brain tumors. The incorporation of a specific precursor (mevalonic acid) in the individual sterols, particularly in brain and brain tumors, was established, and a biosynthetic sequence was described. A new precursor of cholesterol, 4,4-dimethyl-5α-cholesta-8,14 dien-3 β-ol, was identified, and its formation and role were discussed. G. A. LePage (University of Texas, Houston) discussed two examples in which the tumors had suffered partial deletion of catabolic enzymes or changes in enzyme-substrate specificity. In one case, the alpha-enomer of a fraudulent nucleoside was inert in mouse bone marrow but was phosphorylated to the active nucleotide form in some neoplastic tissues. Neoplastic tissues that phosphorylated the nucleoside, alpha-2′-deoxythioguanosine, were responsive to treatment with this nucleoside. In a second case the analogs of adenosine, arabinosyladenine, and xylosyladenine were carcinostatic in some neoplasms. Evidence was obtained for variation in the relative rates of deamination of ribosyladenine, arabinosyladenine, and xylosyladenine from one species to another and from one tumor to another within a species.

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Notes

1. The full text of the papers, edited by the chairman of the conference, George Weber, will be published in the spring of 1970 as volume 8 of *Advances in Enzyme Regulation* (Pergamon, New York and Oxford, in press).
2. The conference was sponsored by the Indiana University School of Medicine, Burroughs-Wellcome and Co., Hoffman-LaRoche, Eli Lilly and Co., and the Squibb Institute for Medical Research.

Courses

Summer Institute on Surtsey, 15 June–1 July. An interdisciplinary course to study the geological, geochemical, geophysical, biological, and ecological implications of the new volcanic island, Surtsey, and selected areas of Iceland. Is intended for university teachers and research workers. Financial support is available for 14 participants. *Deadline for receipt of applications*: 1 March. (Prof. James W. Skehan, S.J., Department of Geology and Geophysics, Boston College, Chestnut Hill, Mass. 02167)

Field Ion and Field Emission Microscopy, Gainesville, Fla., 23–27 March. Among the subjects to be covered are geometry of surfaces and computer techniques, electronic structure of surfaces, field electron emission, field ionization and image formation, field evaporation, grain boundaries and interfaces, metallurgical applications, and atomic order. Travel and subsistence allowances and/or tuition waivers have been made available by the National Science Foundation. (Dr. J. J. Hren, Department of Metallurgical and Materials Engineering, University of Florida, Gainesville 32601)