Meetings

Enzyme Regulation in Mammalian Tissues

The special symposium lecture of Sir Hans Krebs (Oxford, England) on the effects of ethanol on the metabolic activities of the liver was a highlight of the Sixth International Symposium on Regulation of Enzyme Activity and Synthesis in Normal and Neoplastic Tissues, held at Indiana University School of Medicine in Indianapolis, 2 and 3 October 1967. Sir Hans described the correlations between the biochemical disturbances and the clinical manifestations of alcohol intoxication. In the liver, the ratios of nicotinamide-adenine dinucleotide to its reduced form ($NAD : NADH_2$) in the cytoplasm and mitochondria are of importance to the control of metabolic processes, and they are regulated and maintained within relatively narrow limits. Alcohol upsets the regulation of the ratios in the cytoplasm, and this might be a major factor in the pathogenesis of alcoholic liver disease.

At the opening session, the regulation of carbohydrate and lipid metabolism was discussed. P. B. Garland (Bristol, England) reported that palmitoyl carnitine oxidation in isolated hepatic mitochondria inhibited citrate synthase, pyruvate dehydrogenase, and NAD- and NADP-specific isocitrate dehydrogenases, and that it activated pyruvate carboxylation and acetoacetate synthesis. N. R. Marquis (Mead-Johnson) described how fatty acid synthesis in rat liver extract proceeds at a low rate in the absence of citrate or other acetyl-coenzyme A carboxylase activators. Addition of carnitine further decreased lipogenesis, whereas citrate stimulated it. Palmitoyl coenzyme A inhibited certain key enzymes of lipogenesis and glycolysis.

In a liver perfusion system, the stimulatory effect of oleic acid on gluconeogenesis with lactate as substrate was abolished by D(+)-decanoyl carnitine (an inhibitor of palmitoyl carnitine transferase). This effect suggests that gluconeogenesis was controlled by the rate of oxidation of fatty acids (J. R. Williamson, Johnson Research Foundation). The autoregulation of glucose metabolism was studied in the perfused, diabetic rat liver, and there was an increase in glucose utilization with high glucose concentrations which appeared to be at variance with the properties of glucose-phosphorylating enzymes (J. Ashmore, Indiana University).

In a liver supernatant fluid system, acetyl coenzyme A inhibited glucokinase and pyruvate kinase, but did not affect hexokinase or phosphofructokinase (G. Weber, Indiana University). Acetyl coenzyme A is an end product of degradation of free fatty acid that, in turn, is an end product of glucose catabolism; therefore, inhibition of the three key glycolytic enzymes by free fatty acids and the subsequent reinforcement of the inhibition of glucokinase and pyruvate kinase by acetyl coenzyme A are called sequential feedback inhibition.

In human-system studies, a model was proposed to account for the observed bidirectional effects by fructose diphosphate on the velocity of human pyruvate kinase I as resulting from interactions between two or more catalytic sites on this enzyme (R. D. Koler, University of Oregon). In fasted man, the brain adapts to ketoacid utilization while the rate of hepatic gluconeogenesis diminishes (G. F. Cahill, Jr., Harvard Medical School). In these conditions, the kidney contributed a significant proportion of glucose synthesis, this function being related to the rate of ammoniagenesis. The overall decreased nitrogen excretion and brain adaptation permit survival during prolonged periods of starvation.

Cortisol treatment and starvation in rats resulted in a twofold increase in the gluconeogenic capacity of kidney cortex slices, when supplemented with an excess of pyruvate or succinate (W. Seubert, Frankfurt am Main, Germany). The increased pyruvate and phosphoenolpyruvate carboxylases were thought to account for the increased gluconeogenic capacity observed. Rats receiving cortisone injections exhibited enhanced activity of nuclear DNA-dependent RNA polymerase (O. Barnabei, Ferrara, Italy). Also present was a reduced autohydrolysis of pulse-labeled microsomal and of total liver RNA. Control both of synthesis and of breakdown of RNA may influence the response of liver to glucocorticoids.

Evidence on the regulatory action of vitamin K suggests that the messenger RNA for prothrombin is present in the vitamin K–deficient chick, but is not available for translation (R. E. Olson, St. Louis University). All forms of RNA were more rapidly enriched with isotope in the vitamin K–deficient than in normal chick. Vitamin K may have effects upon RNA metabolism that are unrelated to its effect on prothrombin synthesis. N. Katunuma (Tokushima, Japan) showed that fetal liver contained both the liver and kidney types of glutaminases; however, the kidney type gradually decreased and disappeared from the liver in 1 week after birth.

In studying regulation through enzyme activation, E. G. Krebs (University of Washington) observed that the activity of muscle phosphorylase kinase is enhanced by calcium ions through two mechanisms. Of these two calcium effects, the direct requirement by phosphorylase for calcium may be the basis for a control mechanism involved in the coupling of muscle contraction to glycogenolysis. E. R. Stadtman (National Heart Institute) reported that glutamine synthetase activity in *Escherichia coli* is controlled by four mechanisms: (i) repression and derepression of enzyme synthesis; (ii) cumulative feedback inhibition by eight end products of glutamine metabolism; (iii) modulation of the kinetics, divalent ion specificity, and feedback effector responses by enzymic adenylation and deadenylation of glutamine synthetase; and (iv) control of glutamine synthetase activity by variations in the ratios of adenosine triphosphate, Mn^{++}, and nucleoside triphosphates.

A model was presented by D. E. Koshland, Jr. (University of California, Berkeley) to explain cooperative effects in proteins, such as hemoglobin and feedback enzymes. The model involves sequential changes in conformation as ligand is bound, and the efficiency of transmission of the effect in one subunit to a neighboring subunit may vary. A final conformational state depends on the protein and the ligand bound, and, consequently, there may be diversity in the final conformational states with different ligands.

Dihydrofolate reductase was explored, not only as a model drug receptor, but also to elucidate the factors

which contribute to the selectivity for certain neoplasms of drugs known to inhibit this enzyme (C. A. Nichol, Roswell Park Memorial Institute). Drug sensitivity is related to capacity for cellular uptake of amethopterin in tumor systems, and tissues vary with respect to the tetrahydrofolate-requiring pathways most vital to their growth.

The formation of tetrahydrofolate by dihydrofolate reductase is subject to intricate regulatory control (J. J. Burchall, Wellcome Research Laboratories). Since the formation of purines and pyrimidines is extensively controlled, the presence of an additional set of regulatory devices in the folate pathway may function to divert a limited supply of the coenzymes to the critical requirements of the cell. In human leukemia, folate metabolism provides an attacking point to rational chemotherapy (J. R. Bertino, Yale University). In man, an increase in dihydrofolate reductase accompanies treatment with methotrexate in both normal and leukemic leukocytes, and in erythrocytes, within a few days after treatment is started. This increase of enzyme activity with methotrexate represents a type of cofactor induction.

C. G. Smith (Squibb Institute for Medical Research) analyzed the role of cyclic adenosine monophosphate (AMP) in metabolic regulation. Evidence implicating cyclic AMP in a variety of hormone actions was outlined by R. W. Butcher (Vanderbilt University), who compared these results with the effects of cyclic guanosine monophosphate (GMP), which is the only other cyclic 3′,5′-nucleotide identified in nature. The two nucleotides differ with respect to hormonal factors (hypophysectomy, thyroidectomy, adrenalectomy, and administration of thyroxine, hydrocortisone, or glucagon), which alter their excretion in the urine.

Gluconeogenesis from lactate in the perfused liver is stimulated by glucagon and catecholamines, an effect which appears to be mediated by cyclic AMP. Insulin deficiency causes an elevation of cyclic AMP in the liver in vivo, which can be reversed by insulin treatment (J. H. Exton, Vanderbilt University).

The hormonal and nonhormonal control of glycogen synthesis depends on the regulation of transferase phosphatase and kinase (J. Larner, University of Minnesota). The two forms of the transferase enzyme are interconverted by enzymes which catalyze the phosphorylation and dephosphorylation of the two forms of transferase. The site of the nonhormonal control of glycogen synthesis is identified as the phosphatase, whereas the site of the hormonal control by insulin and epinephrine is the kinase.

In the direction and magnitude of its response to glucose and insulin, rat liver adenylate kinase resembles the key gluconeogenic enzymes, whose activities are decreased by insulin administration (S. Weinhouse, Temple University). This newly discovered control of adenylate kinase may have a regulatory effect on gluconeogenesis.

Y. Miura (Chiba, Japan) reported that DNA-dependent RNA polymerase is found both in chromatin and nucleolar fractions of normal rat liver cells, as well as in rat ascites hepatoma cells. Nucleolar RNA polymerase was more sensitive than chromatin RNA polymerase to the administration of steroid hormone. In hepatoma cells, chromatin RNA polymerase was rather insensitive to the steroid hormone. The important problem of energy regulation in rat liver and hepatomas was explored by S. Weinhouse. He pointed out that if competition for adenosine diphosphate (ADP) plays a part in regulating glycolysis in hepatomas, the low activities of pyruvate kinase in the well-differentiated tumors should favor utilization of ADP by the respiratory acceptor system, and this would lower glycolysis further. In contrast, the high pyruvate kinase levels of the poorly differentiated, rapidly growing tumors should favor preferential utilization of ADP by this enzyme and this should enhance glycolysis. He suggested that the characteristically high glycolytic activity of the rapidly growing tumors may in general be attributable to low levels of respiratory ADP acceptor system in combination with high pyruvate kinase activity.

The symposium was sponsored by Indiana University School of Medicine, Burroughs Wellcome and Co., Hoffmann-LaRoche, Eli Lilly and Co., Merck Sharp & Dohme, Squibb Institute for Medical Research, and the Upjohn Co. The full text of the papers, edited by the chairman of the conference, George Weber, will be published in the spring of 1968 as volume 6 of *Advances in Enzyme Regulation* (Pergamon Press). Volumes 1 through 5 of this series of conferences on enzyme regulation in mammalian systems presented the proceedings of the previous five symposia.

GEORGE WEBER

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Calendar of Events

National Meetings

April

20–25. American **Ceramic** Soc., 70th annual, Chicago, Ill. (R. S. Sheldon, 4055 N. High St., Columbus, Ohio 43214)

21–22. American **Broncho-Esophagological** Assoc., Hollywood, Fla. (J. R. Ausband, Bowman Gray School of Medicine, Winston-Salem, N.C. 27103)

21–22. American Soc. for **Artificial Internal Organs**, Philadelphia, Pa. (P. M. Galletti, Div. of Biological Medical Science, Brown Univ., Providence, R.I. 02912)

21–24. American **Oil Chemists** Soc., Memphis, Tenn. (Administrative Assistant, The Society, 35 E. Wacker Drive, Chicago, Ill. 60600)

21–24. American **Orthopaedic** Assoc., Boca Raton, Fla. (S. W. Banks, Executive Secretary, 29 E. Madison St., Chicago, Ill. 60602)

21–24. **Radiation Research** Soc., 16th annual, Houston, Tex. (F. Smith, Biology Dept., American Univ., Washington, D.C. 20016)

21–24. Society of **Head and Neck Surgeons**, Los Angeles, Calif. (H. W. Baker, 2250 NW Flanders St., Portland, Ore. 97210)

21–25. American Assoc. of **Cereal Chemists**, 53rd annual, Cincinnati, Ohio. (Executive Secretary, The Association, 1955 University Ave., St. Paul, Minn. 55104)

21–25. **Paper Coating** Conf., Miami Beach, Fla. (K. G. Chesley, Technical Assoc. of Pulp and Paper Industries, 360 Lexington Ave., New York 10017)

21–26. American **Laryngological, Rhinological and Otological** Soc., Hollywood Beach, Fla. (V. R. Alfaro, 916 19th St., NW, Washington, D.C. 20006)

22–23. **Chemical and Petroleum** Instrumentation Symp., 9th natl., Wilmington, Del. (E. M. Brandle, Leeds and Northrup, 2625 Concord Pike, Wilmington 19803)

22–24. American Assoc. of **Thoracic Surgery**, Pittsburgh, Pa. (A. Hanvey, 311 Carondelet Building, St. Louis, Mo. 63105)

22–24. Association of **Iron and Steel Engineers**, spring conf., St. Louis, Mo. (Managing Director, The Association, 1010 Empire Building, Pittsburgh 22, Pa.)

22–25. American Assoc. of **Petroleum Geologists**, Oklahoma City, Okla. (E. P. Kerr, Jr., Mobil Oil Co., Box 1828, Oklahoma City 73101)

22–25. American College of **Obstetricians and Gynecologists**, 16th annual clinical mtg., Chicago, Ill. (D. C. Sommers, 79 W. Monroe St., Chicago 60603)

22–25. American **Industrial Health** Conf., San Francisco, Calif. (C. D. Bridges, Industrial Medical Assoc., 55 E. Washington, Chicago, Ill. 60602)

22–25. American **Physical Soc.**, Washington, D.C. (W. W. Havens, Jr., Columbia Univ., New York 10027)

22–26. American Soc. of **Tool and Manufacturing Engineers**, Detroit, Mich. (General Manager, The Society, 10700 Puritan Ave., Detroit, Mich.)

22–27. American Acad. of **Neurology**, 20th annual, Chicago, Ill. (S. A. Nelson, 4005 W. 65 St., S., Minneapolis, Minn.)