Meetings

Physics and Nondestructive Testing

The fundamental physics of the various methods and techniques used in evaluating material properties by nondestructive testing was the main theme of a symposium sponsored by the Southwest Research Institute, San Antonio, Texas (1–3 October 1963).

The objectives of nondestructive testing can be achieved only by knowing how and why materials fail. In this symposium papers dealt with the mechanisms for initiating cracks, and the role of flaws in the fracture of materials.

Ultrasonics has been used to investigate the physical properties of materials such as metals, semimetals, magnetic materials, semiconductors, ferrites, and insulators. Experiments were described for measuring properties, such as the magnetoacoustic effect, superconductivity, the effect of the magnetic field on sound velocity, dislocation damping, a depletion layer transducer, the ultrasonic amplifier, the acoustic-electric effect, the temperature dependence of ultrasonic waves in quartz, and measurement of internal stress in magnetic materials. A technique for simultaneously measuring both stress and strain by ultrasonic techniques has also been developed. Acoustic emission, the generation of elastic pulses within the material during deformation, was pointed out as another technique for studying materials and material properties. There is a possibility that acoustic emission may have a bearing on fatigue.

In discussions on the Mössbauer effect, the theory, as well as the application of this technique to materials and material properties, was noted. Specific problems dealt with measurements of nuclear properties, crystalline properties, interaction of nuclear and crystalline properties, magnetic effects, lattice dynamics, annealing of steel, study of glass, surface state, chemical bonding and complex ions, environmental sensors, velocity, and acceleration.

Developments during the past 4 or 5 years in solid-state detectors have pointed up both the advantages and limitations of these types of detectors. A review and discussion of nuclear activation analysis and its applications to measurement of submicrogram amounts of materials was discussed.

In the field of radiation, experiments using a 10- or 25-Mev accelerator and low-energy micro-focus x-rays were noted.

It was announced that the 1964 symposium on physics and nondestructive testing will be held 29 September to 1 October in Dayton, Ohio. The proceedings of the 1963 symposium are to be published and the price is $20 per volume. For further details contact Warren J. McGonnagle.

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Enzyme Regulation in Mammalian Tissues

The regulation of enzyme activity and synthesis in mammalian tissues were themes of an international symposium held 30 September to 1 October 1963 at Indiana University School of Medicine, Indianapolis. Since the theories that have been developed on the synthesis of enzymes in microorganisms are not necessarily applicable to the mammalian enzymes, this meeting afforded an opportunity to exchange ideas and clarify many problems unique to enzyme regulation in mammalian organisms. The regulation of enzymes in the liver was highlighted as a background to the understanding of the apparent failure of regulation of enzymes and metabolic pathways in liver cancer.

Concerning analysis of the metabolic control of gluconeogenesis through enzyme regulation, G. Weber (Indiana University) and H. A. Lardy (University of Wisconsin) discussed the regulation of gluconeogenesis through primary synthesis of key gluconeogenic enzymes. M. F. Utter (Western Reserve University) and Sir Hans A. Krebs (University of Oxford, England) outlined the regulatory effect of metabolites and enzyme activation in governing the rate of gluconeogenesis. In discussing the strategic steps of gluconeogenesis Weber showed that the activity of enzymes participating in gluconeogenesis markedly increases when gluconeogenesis is induced by injection of corticoid hormones, alloxan diabetes, or glycogen storage disease. This increase was inhibited by actinomycin, puromycin, or ethionine, indicating that new enzymes are synthesized in certain stages of gluconeogenesis. Weber also reported that when triamcinolone was administered there was an increase in the activities of glucose-6-phosphatase and fructose-1,6-diphosphatase within 4 to 6 hours. That insulin counteracts the increase in gluconeogenic enzymes induced by corticosteroid hormones suggested that insulin might act as a biological repressor of a sequence of gluconeogenic enzymes genetically located on the same operon.

Lardy demonstrated that phosphoenolpyruvate carboxykinase was markedly increased under gluconeogenic conditions, such as fasting, glucocorticoid administration, or in diabetes induced by alloxan, pancreatectomy, or mannoheptulose. The changes in this enzyme activity appear to be the result of new protein synthesis as demonstrated by the inhibition by ethionine, puromycin, and actinomycin. In contrast, the malic enzyme did not respond to gluconeogenic conditions, but markedly increased after administering insulin.

Utter presented evidence concerning the postulated mechanism for the catalysis of phosphoenolpyruvate formation, the steps being the formation of oxalacetate from pyruvate and the production of phosphoenolpyruvate from this compound. He found that pyruvate carboxylase was cold labile, a factor which had retarded progress in this field until this property of the enzyme was discovered. Utter also showed that the amount, distribution, and properties of pyruvate carboxylase and phospho-

enolpyruvate carboxykinase are consistent with the theory that these enzymes function in the pathway of gluconeogenesis. A unique feature of pyruvate carboxylase is its absolute dependence on, and its high affinity for, acetyl coenzyme A. The data suggested that a control mechanism of oxalacetate synthesis, and possibly of gluconeogenesis, depends on the regulation of pyruvate carboxylase activity by acetyl CoA.

In a special symposium lecture Krebs reviewed the regulation of the rate of gluconeogenesis in animal tissues. The regulatory mechanisms affect the activity of fructose-1,6-diphosphatase, which may be blocked by combined inhibitory effects of fructose-1,6-diphosphate and adenosinemonophosphate. The addition of lactate removed the block by causing the dismutation reaction: lactate + triose-P $\rightleftharpoons$ pyruvate + alpha-glycero-P, with a resulting accumulation of alpha-glycero-P instead of fructose-1,6-diphosphate. Another control point occurs at the conversion of pyruvate to oxalacetate and in turn to phosphoenolpyruvate, which is greatly stimulated by acetoacetate and other precursors of acetyl CoA. Acetoacetate does not contribute to the net synthesis of carbohydrate; thus its effects on the rate of gluconeogenesis must be caused by an action on an enzyme system of gluconeogenesis. Experimental observations support the work of Utter that acetyl CoA activates pyruvate carboxylase. A further control point in gluconeogenesis is the availability of the reduced nicotinamide adeninedinucleotide (NADH) required for the triosephosphate dehydrogenase reaction. Krebs pointed out that oxalacetate inhibited gluconeogenesis in kidney cortex slices and that this inhibition was paralleled by a decrease in NADH as a result of the rapid reduction of oxalacetate to malate.

O. Wieland (University of Munich) analyzed the regulation of acetyl CoA metabolism, stressing the key role of citrate synthase. This enzyme was markedly inhibited by palmityl-CoA in a noncompetitive way with respect to acetyl CoA. Since palmityl-CoA lowered the affinity of citrate synthase for oxalacetate, Wieland suggested that this might be regarded as a type of regulation which would provide control of the Krebs cycle with only small changes in the concentrations of oxalacetate and palmityl-CoA.

The isotope exploration of gluconeogenesis was evaluated by J. Ashmore (Indiana University). He showed that the labeling pattern of glucose produced in liver slices that had been incubated with pyruvate-2-C^{14} is consistent with pyruvate carboxylation and equilibration of the resulting compounds in the dicarboxylic acid shuttle. Increased incorporation of C^{14} from labeled CO_2 into glucose occurs in vivo and in vitro in rats under conditions of increased hepatic glucose formation. For instance, in alloxan diabetes the incorporation of $C^{14}O_2$ into glucose was increased three- to sevenfold. Ashmore also observed the increase in phosphoenolypruvate carboxykinase activity with no alteration in the activity of the malic enzyme. Acute insulin deficiency induced in rats by injecting anti-insulin serum markedly increased phosphoenolpyruvate carboxykinase activity of the liver; at the same time there was an increased incorporation of $C^{14}O_2$ or C^{14}-pyruvate.

F. Rosen and C. A. Nichol (Roswell Park Memorial Institute) emphasized the importance of adequate amino acid pools in conditioning the response to corticosteroid or diet, of such enzymes as serine dehydrase, tryptophan pyrrolase, alanine and tyrosine transaminases, and urea cycle enzymes.

Problems of gluconeogenesis in man were explored by G. F. Cahill, Jr. (Harvard Medical School). Emphasizing the role of the kidneys in maintaining blood sugar, he pointed out that an agent which would depress renal gluconeogenesis without affecting the rate of hepatic inflow or peripheral outflow would theoretically cause symptomatic hypoglycemia within an hour. In patients under hypothermia, analysis of liver glucose and lactate gave values within 20 percent of blood glucose and lactate concentrations, indicating that significant glycogenolysis did not occur under these conditions. Such metabolic stability and viability of liver, when cooled, provided opportunity for extensive hepatic surgery, including homotransplantation in man. Cahill also discussed mechanisms of important syndromes, such as hypoglycemias which are caused by ethanol and various sugar phosphates.

A remarkable agreement concerning the regulation of hepatic glucokinases was reached by S. Weinhouse (Temple University), A. Sols (Madrid, Spain), and V. S. Ilyin (University of Leningrad). Weinhouse showed that the livers of rat, mouse, rabbit, guinea pig, and hamster contained a soluble glucokinase that differs from previously described phosphotransferases in having a high K_m for glucose and in responding adaptively to hormonal and dietary conditions. Glucokinase was decreased from 25 to 33 percent of its normal level in alloxan diabetes, whereas hexokinase did not change. When rats with alloxan diabetes were given insulin, hepatic glucokinase activity increased to normal in less than a day and remained normal until insulin was withdrawn, when it gradually returned to the low diabetic level. Sols showed that the insulin induction for glucokinase in diabetic rats did not involve an interplay of activators or inhibitors but was due to fresh synthesis of the enzyme.

Thus, at this meeting, two opposite effects of insulin on hepatic enzyme induction were revealed: (i) inhibition of synthesis of gluconeogenic enzymes, and (ii) induction of glucokinase which may be considered the first step of the metabolic pathway running opposite to gluconeogenesis.

Advances in the discovery of feedback mechanisms acting on mammalian enzymes were presented. M. D. Siperstein (University of Texas) pointed out that the feedback inhibition operating in cholesterol metabolism may be localized at the conversion of beta-hydroxy-beta-methylglutaryl CoA to mevalonic acid. Cholesterol feeding inhibited mevalonic acid synthesis in both cell-free systems and liver slices in a degree sufficient to account for the depression of cholesterol synthesis. He also demonstrated the operation of the feedback system in human liver biopsy specimens. E. Bresnick (Baylor University) showed pronounced inhibition of liver aspartate transcarbamylase and dihydro-orotase by pyrimidines, pyrimidine analogs, and purine derivatives. He emphasized the role that thymidine and purine deoxyribonucleosides play in regulating the fresh synthesis of pyrimidines. E. Scarano (Naples, Italy) showed that deoxycytidylate aminohydrolase has at least one allosteric site. By various allosteric effects this enzyme can regulate the pool of pyrimidine deoxynucleotides. H. C. Pitot (University of Wisconsin) found that glucose repression, a mechanism well known in microbial enzyme regulation, also repressed the dietary induction of threonine dehydrase and ornithine transaminase in rat liver. These enzymes were also inhibited when actinomycin was given at the beginning of the induction. However, in later stages of enzyme induction this antibiotic had

no effect, suggesting that a sufficient amount of RNA template had been formed for enzyme synthesis. Regulation of glycolysis was discussed by J. V. Passonneau (Washington University) in terms of regulating phosphofructokinase activity; she postulated a number of sites on the enzyme that were distinct from inhibitory and substrate sites previously documented.

Various mechanisms of enzyme induction were discussed by W. E. Knox (Harvard Medical School), who also reported his recent work on tyrosine transaminase. In young rats this enzyme increased after the administration of tyrosine and ascorbic acid prevented the elevation of the enzyme produced by either tyrosine or extra protein feeding. He discovered that older rats failed to respond to these stimulations and suggested that the effects had been missed in the past because of the age limitation of the response and its delayed time course. Knox classified the enzyme-induction mechanisms into "hormone-type" and "substrate-type" induction processes. The hormone (for example, glucocorticoid) increases the amount of certain limiting RNA moieties and thus causes an increased synthesis of the enzyme. He suggested that the "substrate-type" induction results from sequestering an enzyme in a combined form that cannot be degraded.

The cofactor mediating regulation of liver enzyme levels was discussed by O. Greengard (Institute for Muscle Disease); she showed that the amount of rat liver apocysteine sulfinate decarboxylase and tyrosine transaminase can be influenced in vivo by the concentration of their cofactor. Pyridoxine administration in intact or adrenalectomized rats caused a 200-percent increase in hepatic tyrosine transaminase activity in 4 hours. Greengard showed that this increase reflected a rise in the concentration of the apoenzyme and that puromycin interfered with this cofactor-induced increase. She contrasted the mechanisms in cofactor induction with those of hormonal regulation of enzyme concentrations. A. E. Harper (M.I.T.) showed that ammonia toxicity in the rat depends on the activities of enzymes taking part in urea formation. E. Hirschberg (Columbia) discussed regulatory effects of glutamic dehydrogenases in rodent liver.

Aspects of the production, development, and biological properties of transplantable hepatomas were described by H. P. Morris (Bethesda) who has produced these tumors by chemical carcinogenesis. The correlation of growth rate with the extent of enzymatic alterations was documented by other investigators. G. Weber reported a gradual decrease in key enzymes of gluconeogenesis (glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, and malic dehydrogenase) as the growth rate of the hepatomas increases. The aforementioned enzymes were markedly decreased or completely absent in the rapidly growing tumors. J. Ashmore reported that with increasing growth rate there was an increase in lactate production, oxidation of glucose at C-1 and C-6, and an incorporation of amino acids into protein (alanine, aspartate, glycine, serine, isoleucine, and valine). In contrast, there was a gradual decrease of glucose production from pyruvate with complete failure of this gluconeogenic pathway in the rapidly growing tumors from which the key gluconeogenic enzymes were missing. G. P. Wheeler (Southern Research Institute, Birmingham) showed that with the increasing growth rate there was a decrease in purine catabolism and a gradual increase in the extent of incorporation of formate-C^{14} into the purines of both DNA and RNA.

J. S. Roth (University of Connecticut) reported that thymidylate phosphatase activity was inversely correlated with growth rate, the activity was greatest in slowly growing hepatomas and lowest in the rapidly growing tumors. In contrast, thymidine kinase showed a direct correlation with growth rate, exhibiting low activity in the slowly growing tumors, but high activity in the rapidly growing hepatomas. All investigators pointed out that there were a number of enzymes and metabolic parameters that were either high or low in all tumors or showed no correlation with the growth rate.

V. R. Potter (University of Wisconsin) suggested that certain differences between normal and tumor tissues may be related to growth rate, to invasiveness, to metastatic tendency, or to none of the components of malignancy. The cancer investigators agreed that the exploration of hepatomas of different growth rates permits valuable advances regarding the molecular basis of the biological behavior of liver tumors.

The symposium was sponsored by the Damon Runyon Memorial Fund, Inc., and the Indiana University School of Medicine. Chairmen of the sessions were Sir H. A. Krebs, J. W. Wilson (Brown University), C. F. Cori (Washington University), V. R. Potter, W. E. Knox, and S. Weinhouse. The full text of the papers, edited by George Weber, will be published in the spring of 1964 as volume 2 of *Advances in Enzyme Regulation* (Pergamon Press, Oxford; Macmillan, New York). Volume 1 of this series of symposia on enzyme regulation in mammalian tissues was published in 1963 and presented the proceedings of last year's meeting.

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Forthcoming Events

April

13–15. Institute of **Environmental Sciences**, annual, Philadelphia, Pa. (J. Breen, RCA Bldg., 10-1-2, Camden 2, N.J.)

13–15. **Microelectronics**, 3rd annual symp., St. Louis, Mo. (T. F. Murtha, P.O. Box 4104, St. Louis, Mo. 63136)

13–16. American Acad. of **General Practice**, Atlantic City, N.J. (M. F. Cahal, Volker Blvd. at Brookside, Kansas City 12, Mo.)

13–16. **Industrial Health**, conf., Pittsburgh, Pa. (American Industrial Health Conf., 55 E. Washington St., Chicago, Ill.)

13–16. **Industrial Medical** Assoc. and American Assoc. of **Industrial Nurses**, Pittsburgh, Pa. (C. D. Bridges, 55 E. Washington St., Chicago, Ill. 60602)

13–16. American **Radium** Soc., White Sulphur Springs, W. Va. (J. J. Stein, U.C.L.A. Medical Center, Los Angeles 24, Calif.)

13–17. **Fluid Power**, intern. conf. and exhibition, London, England. (Secretary of the Conference, The Tower, 229-243 Shepherds Bush Rd., Hammersmith, London, W.6)

14–16. **Power** Conf., Chicago, Ill. (W. A. Lewis, Illinois Inst. of Technology, Chicago)

14–18. Primary Disorders of **Heart Muscle** (by invitation), CIBA Foundation symp., London, England (CIBA, 41 Portland Pl., London, W.1)

14–18. **Mathematical Logic**, conf., Oberwolfach, Germany. (M. Barner, Mathematisches Forschungs-institut, Hebelstr. 29, 78 Freiburg im Breisgau, Germany)

15–17. **High Energy Physics**, conf., Chilton, England. (Inst. of Physics and the Physical Soc., 47 Belgrave Sq., London S.W.1, England)

15–17. **Ophthalmological** Soc. of the United Kingdom, annual, Dublin, Ireland. (Secretariat, 47 Lincoln's Inn Fields, London, W.C.2, England)

15–18. British **Paediatric** Assoc., annual, Scarborough, England. (E. W. Hart, Inst. of Child Health, Hospital for Sick Children, Great Ormond St., London, W.C.1)