Meetings

Biotransformations and Fermentations

Traditionally considered as completely separate topics, the metabolic disposition and formation of a variety of organic compounds in microbial, plant, and mammalian systems constituted the subject of a symposium, "Biotransformations and Fermentations," at the 14th annual meeting of the American Society of Pharmacognosy held at Jekyll Island, Georgia, 15 through 20 July 1973.

A talk on microbial transformations of antibiotics was presented by O. K. Sebek (Upjohn). Microbial modification of antibiotics has resulted in the preparation of many new semisynthetic antibiotics. Examples of three clinically important microbial reactions (*N*-acetylation, O-phosphorylation, and adenylation) through which antibiotics are inactivated by bacteria which carry R factors were discussed. The identification of products of such reactions has made it possible to chemically synthesize modified antibiotic derivatives like the kanamycins which are resistant to inactivation. Microorganisms alter antibiotics in a variety of ways including hydroxylation, hydrolysis, demethylation, carboxymethylation, reduction, and transglycosylation. With few notable exceptions, the metabolic products of antibiotics are inactive or are biologically inferior to the parent compounds. The preparation of new antibiotics by incubating analogs of antibiotic subunits with the antibiotic-producing organism and by mutant strains was also discussed.

D. R. Brannon (Eli Lilly Research Laboratories) considered aspects of screening fermentations for pharmacologically active compounds other than antibiotics. In particular, emphasis was given to the elaboration by microorganisms of compounds that function as specific enzyme inhibitors or stimulators. Principles and techniques involved in the operation of screening for pharmacological activities from microbial cultures were presented, along with several possible enzyme analytical models which may be used to monitor the presence or absence of pharmacodynamic principles in fermentations.

Aspects of the biochemistry of penicillin and cephalosporin fermentations were presented by A. L. Demain (Massachusetts Institute of Technology). The biosynthesis of hydrophobic penicillins such as benzylpenicillin by *Penicillium chrysogenum* with the tripeptide, α-(L-α-aminoadiply)-L-cysteinyl-D-valine and isopenicillin N was discussed. Studies indicate that 6-aminopenicillanic acid (6-APA) is a shunt metabolite produced by hydrolysis of isopenicillin N when hydrophobic side chain precursors are unavailable. L-α-Aminoadipate is an intermediate in fungal biosynthetic pathways to lysine and to penicillins involving a branched pathway. Lysine causes diminution of penicillin formation, presumably by inhibition of homocitrate synthase, the first enzyme of the early common pathway to the amino acid and to penicillin. It was indicated that penicillin biosynthesis is also controlled by catabolite repression by glucose and feedback inhibition by penicillin itself. Although *Cephalosporium acremonium* produces hydrophilic penicillin N, 6-APA does not appear to be an intermediate in the biosynthesis of penicillin N. This organism also produces cephalosporin C, a β-lactam antibiotic with a D-aminoadipyl side chain. Precursors are L-aminoadipate, L-cysteine, L-valine, and acetate. The nucleus of the antibiotic, 7-aminocephalosporanic acid, has never been detected in broths, and is probably not an intermediate. Both D-methionine and norleucine, a nonsulfur analog of methionine, were reported to stimulate biosynthesis of cephalosporin C.

B. E. Ellis (Trent University) reviewed the degradation of aromatic compounds in plants. The metabolic disposition of simple and complex aromatic structures to carbon dioxide was discussed. Catabolic reactions include demethylation, decarboxylation, and fission of the aromatic ring. The degradation of tyrosine occurs by means of the homogentisic acid ring cleavage pathway, by ring cleavage of dihydroxyphenylalanine (dopa), and by ring cleavage of cyanogenic glycosides that were derived from tyrosine. Some enzymes involved in plant catabolism of aromatic compounds have been detected in cell-free extracts of plant tissues, and studies presented indicated that the accumulation of secondary metabolites is probably a dynamic process with a balance being maintained between anabolic and catabolic reactions within the cell.

Biotransformations in plant cell cultures was the topic of W. Steck (National Research Council of Canada, Saskatoon). He cited the fact that, although cell suspension cultures of plant tissues possess the capacity to form secondary products, the rates of biosynthesis are usually low. Methods to enhance biotransformations leading to products have been devised. These include hormonal manipulations, environmental controls, and the use of chemical precursors. Emphasis was placed on the use of exogenous chemical precursors which are transformed by cells to a storage product. Examples were given of the use of precursors such as coumarins, quinoline alkaloids, and cinnamic acid derivatives in suspension cultures of a number of plants.

A stimulating presentation on the control of production of useful products by pituitary tumor cells in culture was made by P. M. Hinkle (Harvard). Results of studies on established clonal strains of rat pituitary tumor cells which secrete either prolactin, growth hormone, or both prolactin and growth hormone into culture medium was discussed. Relatively large quantities of the hormones are synthesized: 5 to 30 μg per milligram of cell protein in 24 hours, or 2 to 15 percent of the total protein synthesized. Such cells are of value in studying factors controlling hormone production. The effects of hydrocortisone, which increases growth hormone synthesis and decreases prolactin production, and of estradiol which has the opposite effect were covered. The hypothalamic releasing factor—that is, thyrotropin releasing hormone (TRH)—also causes cultured cells to increase prolactin production and decrease growth hormone production. The actions of TRH are mediated by specific receptors for the tripeptide, and binding of TRH to cells is conveniently measured by use of tritiated

TRH. One cell line was used to study the biological and binding activities of more than 40 peptide analogs of TRH. The potential utility of the prolactin and growth hormone releasing cell system for testing the activity of physiologically important regulators of prolactin and growth hormone production, in studying the mechanisms of action of these substances in a defined and easily controlled environment, and in providing a system in which to test analogs of steroid, peptide, and alkaloid regulators that might prove useful as pharmacological agents was emphasized.

Developments in the field of drug metabolism were discussed in the presentation, "Biotransformations in mammals—a perspective," by R. E. McMahon (Eli Lilly Research Laboratories). Early work with the metabolite 4-transhydroxyethinylcyclohexylcarbamate, involving its degradation and comparison to known synthetic compounds, required a year for completion. More recently, *d*-propoxyphene metabolism has been studied in man with the aid of combined gas chromatography–mass spectrometry and heavy isotopes in approximately 1 to 2 months. Other examples of the use of analytical technology in drug metabolism studies were given. The structure of mammalian acronycine metabolites was elucidated by mass spectral and nuclear magnetic resonance techniques. Extremely small quantities of material were sufficient for this determination of structure.

In a somewhat unique combination of the fields of alkaloid chemistry and mammalian drug metabolism, S. Teitel (Hoffmann-LaRoche) described research efforts on alkaloids in mammalian systems. Alkaloids presumably form in a stereospecific fashion by enzyme-catalyzed Pichtet-Spengler condensations of amino acids and biogenic amines to form various hydroxy-substituted tetrahydroisoquinolines and tetrahydro-β-carbolines. Condensation products of L-dopa, its two mono-*O*-methyl ethers, L-tryptophan, and its 5-hydroxy derivative with formaldehyde and acetaldehyde were characterized. Additional work with the benzylisoquinolines, including oxidative coupling experiments to form aporphines, was described. The potential for forming these compounds in vivo, as well as the possibility that they possess biological activity, was discussed.

D. M. Jerina (National Institutes of Health) summarized findings from his laboratory and others on the biological formation and disposition of arene oxides. He emphasized that drugs and other environmental chemicals are metabolically activated by the formation of arene oxides which may rearrange to phenols, be hydrated to diols, become conjugated with glutathione, or react with nucleophilic sites on macromolecules. Because arene oxides are highly reactive compounds, it has been difficult to demonstrate that they are enzyme-generated intermediates. Indirect criteria such as the formation of dihydrodiols and mercapturic acid precursors as well as intramolecular migrations accompanying aryl hydroxylation (the NIH shift) have been used to support this mechanism. Chemical and enzymatic factors controlling the formation, lifetime, and disposition of arene oxides within the cells were discussed.

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Genetic Recombination: Genetic, Physical, and Biochemical Aspects

Recent advances in the understanding of genetic recombination have resulted from experimental work in diverse areas of molecular biology. The Symposium on Genetic Recombination, held at the Roche Institute of Molecular Biology (2 to 4 May 1973), brought together scientists whose work represents some of the more important research directions in this field.

In the opening sessions new results obtained from genetic analyses in eukaryotic as well as prokaryotic organisms were considered. These included the analysis of gene conversion in unselected populations of meiotic tetrads of yeast (S. Fogel, University of California, Berkeley) and genetic evidence for a correction mechanism and the formation of hybrid DNA during recombination in *Ascobolus immersus* (J. L. Rossignol, Université de Paris-Sud, Orsay, France). Both systems have the distinct advantage that all the products of a single recombination event can be recovered and analyzed. Genetic analysis of recombination in viruses and prokaryotes indicate that gene conversion (unequal recovery of parental markers) occurs in these systems also (T. Boon, Institut Pasteur, Paris, France) and a molecular mechanism, based on possible repair of "mismatched" bases has been proposed. Whether there are separate enzymes involved in this phenomenon, analogous to those known for ultraviolet repair, remains to be elucidated. The technology available in prokaryotic systems should be adequate to answer this question in the near future.

Other topics in these sessions dealt with what might be called the "genetics of genetic recombination systems." A. J. Clark (University of California, Berkeley) presented results which indicate that there are at least two pathways of general recombination for *Escherichia coli* (revealed by the *rec* mutations A, B, C, F, and L, and *sbc*A) and suggested ways in which they could be interconnected. A separate and distinct system for general recombination is encoded by bacteriophage lambda (J. Zissler, University of Minnesota). A second class of recombination, responsible for the incorporation of viral genetic material into the chromosome of the host, is typified by the site-specific *int-xis* system of lambda, and was discussed by A. Campbell (Stanford University), H. Echols (University of California, Berkeley), R. A. Weisberg (National Institutes of Health), and M. E. Gottesman and S. Gottesman (National Institutes of Health). Zissler has proposed that other genes or sites in the recombination region of lambda, called delta and epsilon, may be involved in site-specific recombinational events. Several workers discussed another lambda gene, gamma, whose product interacts with the host B,C nuclease and which affects both the recombinational and replicative pathways of the viral DNA. The studies of A. I. Bukhari (Cold Spring Harbor Laboratory) on the reaction involved in the integration and excision of the DNA of phage Mu show that this process is only half site-specific. Though the crossover point on the viral DNA is fixed, and presumably contains a site recognized by the Mu inte-