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CHASING NUCLEOTIDES

The U. of Nebraska Chemistry Dept. had a truly fine cadre of chemistry teachers, as I have subsequently realized upon more experience in the real academic world. From an M.S degree in biochemistry under Walter Militzer in 1949, it was my good fortune to be accepted as a student of Van Potter at the U. of Wisconsin McArdle Laboratory for Cancer Research. Potter had the insight that the 4-carbon Krebs Cycle metabolites were precursors of the nucleic acid pyrinridines via orotic acid. He suggested preparing C 14-labeled orotic acid, which I succeeded in doing under the tutelage of Charles Heidelberger, and following its distribution in rat tissues and tumors and pyrimidines of pentose nucleic acid (as RNA was then known as). In the course of this work, I developed a system for anionexchange chromatography which resolved free uridine mono-, di-and tri-phosphates in tissue extracts, as well as other nucleotidcs, The uridine nucleotides were quickly and strongly' labeled from the orotic acid, supporting the idea that orotate is a true intermediate in biosynthesis of RNA . We used this method for resolution of tissue free anions: --"pearl diving" commented Waldo Cohn, who pioneered this ion-exchange chromatography. Hanus Schmitz was excited to discover the small amount of cytidine triphosphate, which was also labeled by the orotic acid. This helped complete the picture of RNA precursors and biosynthesis in animal tissues and tumors, tying it to the synthesis of RNA molecules by purified bacterial enzymes. It also landed me a Ph,D. in Physiological Chemistry, a two year Fellowship from the American Cancer Society and an invitation to work in Prof. Hammarsten's Kemikum I lab at Karolinska Institutet in Stockholm, with Peter Reichard. (They had been the first to show conversion of N15 labeled orotic acid to nucleic acid pyrimidines.)

We found that cytoplasmic enzyme extracts of diverse tissues and tumors; were capable of converting orotic acid and ribose-5-triphosphate to uridine nuc1eotides. We were scooped in this by Arthur Kornberg and associates who also demonstrated this, more elegantly but less comprehensively, We wondered about the mechanism of formation of the cytidine uuc1eotides, and began to work on a spectrophotometric assay in the Cytofysilogi lab of Hans Klenow in Copenhagen.

Returning to McArdle Lab, it was my good fortune to be invited to join (in 1955) Clark Griffin's Biochemistry Dept., in the new U. of Texas M.D. Anderson Hospital and Tumor Inst. created by R. Lee Clark.

Scooped again! L Lieberman, one of Komberg's associates, used a spectrophotometric assay to demonstrate amination of UTP to CTP with requirements for ammonia and ATP, by partially purified enzymes from E.coli. Harold Kammen, Kamala Chakraborty, and I re-examined the reaction with intact tumor cells and soluble enzymes; we found the amide group of glutamine (instead of ammonia) to be required, and --surprisingly-- guanosine nucleotides as cofactor. Even in extracts of E. coli.! This suggests an interesting regulatory system that provides adequate but not excessive amounts of ribotides for RNA synthesis. *CTP* is a critical precursor for the large amounts of

cytosine in ribosomal RNA; UTP is needed for CTP synthesis (as well as a number of other reactions), and both ATP and GTP are necessary to assure a full complement of RNA precursors. Runaway production of CTP is suppressed by a negative feedback effect of CTP itself· The anti-cancer drug DON is a glutamine analog/antagonist; it strongly inhibits CTP and RNA synthesis.

Next the deoxyribonuc1eotides. These are present in growing cells at such low levels as to be undetectable in the presence of 10 times as much ribotides. My colleague, Colleen Moore, and I worked out techniques for selectively absorbing and removing the ribotides on boronate-polyacrylamide gel. "Pearl-diving" with high resolution anion-exchange columns then showed (for instance) the presence of the deoxynucleotides in rapidly growing tumors, regenerating liver, and Sphase cells, but not normal liver. We then discovered cytidine nucleotides could be converted (reduced) to deoxycytidine nucleotides, in animal enzyme extracts. The cytidine nucleotide reductase required NADPH and Fe+++, Later, Moore (with Reichard) went on to show dependence on thioredoxin as well.

We set up fairly rapid multiple assays for production of each of the deoxytides from the corresponding P32-labeled ribotides (selfprepared), using small boronate columns to separate precursors from labeled product. We found a complex regulation system whereby the rate of formation of each of the deoxytides is controlled by the concentrations of other ribotides or deoxytides acting as positive or negative effectors. The function of this system is to maintain the presence of all 4 deoxytides in low but roughly equal concentrations for DNA synthesis. Knowledge of these regulatory effects has helped explain the antigrowth activities of many inhibitors and analogs ofnuc1eosides and nucleotides. For instance: adenosine; hydroxyurea, heterocyclic thiosemicarbazones, and alanosine.

Ming Liau and I explored RNA synthesis in nuc1ei and in nucleoli isolated from rat liver and tumors, using P32 labeled nucleotides. The nucleoli are subnuclear organelles responsible for production of ribosomal RNA, mainly 18 and 28 S preribosomal RNAs, and are also capable of processing and methylating these subunits. Polyl-polyC appears to inhibit the methylases. Interestingly, Aurelie France found the nucleoli show evidence they contain a unique complete independent system of protein synthesis; significance not known.

Academically speaking, I taught courses at Baylor College of Medicine for several years, then spent a good deal of time helping organize the new UT Graduate School ofBiomedical Sciences (even acting as the first registrar!), then teaching a few courses and supervising graduate students and exams towards the Ph.D. Degree.

So, I have chased orotic acid all the way into RNA and DNA, with some glimpses into metabolic regulation and tumor inhibitory mechanisms, Almost all of this work has been supported by the American Cancer Society, for which 1am exceedingly grateful I have tried to repay by serving for a number of years on grant review panels and site visits for the ACS, also by quite extensive participation on the Editorial Board of Cancer Research. The 50 Year recognition by the American Association for Cancer Research is highly treasured by myself and my family.