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JOHN MACHLIN BUCHANAN
1917—2007

A Biographical Memoir by

STANDISH C. HARTMAN AND BRUCE ZETTER

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John M. Buchanan

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BY STANDISH C. HARTMAN AND BRUCE ZETTER

JOHN MACHLIN BUCHANAN—JACK, as he was affectionately known to all his students, colleagues, and friends—was born September 29, 1917, in Winamac, Indiana, and died June 25, 2007, in Burlington, Massachusetts, after a long and notable career as a scientist, mentor, teacher, and administrator. He is survived by his wife, Elsa Nilsby Buchanan, his indispensable source of love and support for 57 years, and his four children and nine grandchildren. More than 80 former graduate and postdoctoral students are indebted to Jack for his personal and invaluable contributions in helping them start their careers. Many of them continued to be his and Elsa's lifelong friends. His influence, through his many students who went on to enjoy significant professional careers in academia and industry, stands as an inheritance that Jack, his family, and all who worked with him may regard with pride.

During his career, Jack was the recipient of many honors. He received the Ely Lilly Award in Biochemistry of the American Chemical Society in 1951 and was a Harvey Society lecturer in 1958. He was elected to the American Academy of Arts and Sciences in 1953 and to the National Academy of Sciences in 1962. He served as secretary of the American Society of Biological Chemists (now the American Society for Biochemistry and Molecular Biology) from 1969 to 1972, and

on the editorial boards of the *Journal of Biological Chemistry*, *Journal of the American Chemical Society, Physiological Reviews, Federation Proceedings, and Journal of Molecular and Cellular Biochemistry*. In 1967 Jack was appointed as the first John and Dorothy Wilson Professor at MIT, a position he held until his retirement in 1988. He held honorary doctorates from the University of Michigan and from his alma mater, DePauw University. In 2006 Jack's students and friends established the John M. Buchanan Lectureship at MIT to endow an annual lecture in the Biology Department to memorialize his life's work, his service to the institute and the profession, and their affection for the person who had so enriched their lives.

Jack candidly judged that his professional reputation most directly rested on his work on purine biosynthesis. It is appropriate as backdrop to Jack's mature period of scientific work to recall his early education and experiences, both as they connected with mentors who influenced his development and as his own decisions along the way brought him to the scientific contributions for which he is well known.

His scientific career spans a period, starting in 1938, that coincides with the emergence and flowering of the field of biochemistry as a central area of mechanistic biology. In an autobiographical chapter he wrote in 1985 for *Comprehensive Biochemistry* volume 36 he notes the pioneering nutritional studies on amino acid metabolism in animals by William C. Rose and Howard B. Lewis in the 1920s that formed the essential background to later intermediary metabolic work, including his own. Lewis would later figure directly in Jack's education. As a graduate student Jack participated in some of the earliest applications of radioisotopic carbon in studying metabolic pathways in vivo. More detailed knowledge of intermediary metabolism, he realized, required moving to in vitro systems, including tissue slices and homogenates, and eventually to the isolation and characterization of individual

enzymes. The foundation laid down by mainstream biochemistry broadly supported the burgeoning fields of molecular biology and cell biology, which increasingly occupied Jack's research in his later years, into the 1970s and 1980s. Jack was ever mindful of the historical roots of his chosen area of science—his graduate students could always expect a question on their prelims that required as much appreciation of history as of science—and so it is appropriate to take note of how the arc of his career so closely tracked the exciting evolution of biochemistry through the middle years of the 20th century.

EARLY SCIENTIFIC STIRRINGS

Jack realized for the first time that science might be an interesting area to pursue during an elective chemistry course he took in high school. His first exposure to research was as a senior undergraduate student at DePauw University in Greencastle, Indiana, where he majored in chemistry and minored in mathematics. He undertook a project on the synthesis of mandelic acid under the tutelage of a young professor, Jesse L. Riebsomer. This project exposed him to the "thrill and frustration" of research, he later recalled, and it led to his first publication, in the *Journal of the American Chemical Society*, as a joint author. He finished his undergraduate work in 1938, faced with the question of "what next?" If Riebsomer's encouragements had prevailed, Jack might have ended up as an organic chemist, headed to Pennsylvania State University on a graduate assistantship. However, that assistantship fell through because of a major fire in the Chemistry Department at Penn State. Jack in the meantime had a nascent interest piqued in a different direction while reading Bodansky's *Physiological Chemistry*.

GRADUATE WORK: MICHIGAN AND HARVARD

A family friend suggested that he look into graduate work at the University of Michigan (his family had moved from Indiana to Kalamazoo, Michigan, during his college years). He took that advice and applied to the Biochemistry Department at the University of Michigan, where he was admitted in the fall of 1938. Professor Howard Lewis was then chair of the department, and although Jack's tenure at Michigan was brief, Lewis's support and friendship lasted for many years. Jack found Professor Lewis to be an inspired lecturer in his highly regarded course in biochemistry. That stimulating experience cemented Jack's future career choice. Lewis's graduate course relied on reading the current literature, which included Cori's work on glycogen synthesis, Hans T. Clarke at Columbia on nitrogen metabolism, and the isotopic tracer studies of Rudolph Schoenheimer and David Rittenberg. These reports, in particular, directly connected with Jack's later research undertakings. He completed a master's degree investigating concanavalin A in 1939. His year at Michigan was rewarding as his first real immersion in biochemistry but also a difficult one because circumstances did not afford him a supporting assistantship.

With Lewis's gracious support, Jack was encouraged to apply to other schools, among them the Department of Biological Chemistry at Harvard Medical School, where A. Baird Hastings was chair. Hastings provided a welcome financial stipend that allowed Jack's full-time dedication to his Ph.D. work and the opportunity to collaborate with and to learn from a large group of superb mentors. His first year was spent mainly on the Harvard University campus in Cambridge, taking courses in chemistry and biophysics and tutoring in biochemistry, the latter aided by an assistantship provided by John Edsell, another lifelong friend. Hastings and his students were part of an interdepartmental group

that had access to a cyclotron as a source of the radioactive isotope ^{11}C. This would be an invaluable tool in their study of gluconeogenesis from lactic acid. In addition to Hastings, the Harvard group included James B. Conant (Harvard's president), George Kistiakowsky, Richard Cramer, Friedrich Klemperer, Birgit Vennesland, and Arthur Solomon. Solomon had constructed a Geiger counter, the first in the Boston area, which was employed for quantitative measurement of the isotope. A characteristic of ^{11}C is its short half-life—about 20 minutes—which meant that metabolic experiments had to be executed start to finish within four hours at the most. Jack, being the youngest member of this collaboration and fairly athletic, served as the gofer between the cyclotron in Cambridge and the lab at the medical school campus across the river in Boston, traveling either by foot or bicycle. These were rather looser times, before the days of strict oversight by the Atomic Energy Commission, which might have frowned on such casual means of transporting "hazardous materials."

Access to ^{11}C came at a propitious time. Hastings's research had examined glycogen synthesis using in part the traditional approach of measuring the increase in liver glycogen after feeding nutrients, such as lactate, to fasting animals. Cramer and Kistiakowsky synthesized 1-^{11}C-lactic acid and 2,3-^{11}C-lactic acid; these led to the rapid labeling of liver glycogen in rats. Significantly, the amount of label arising from the carboxyl group of lactic acid was only half that incorporated from the 2,3-labeled precursor. These results were consistent with the idea proposed earlier by H. G. Wood and C. H. Werkman, and by E. A. Evans and L. Slotin that the three-carbon compound pyruvate (and indirectly its relative, lactate) combines with CO_2 to form four-carbon intermediates of the Krebs citric acid cycle that equilibrate among symmetrical compounds (e.g., fumarate). Random loss of one of the two carboxyls of fumarate to form

a three-carbon precursor of glucose (which is now known to proceed through oxaloacetate and phosphoenolpyruvate) would reduce by half the amount of label arising from the carboxyl of lactic acid relative to the other two positions. As a corollary, Vennesland realized that labeled carbon dioxide should show up in glycogen arising from unlabeled lactate, as it equilibrates through this same series of reactions. When the experiment with $^{11}CO_2$ was conducted, this prediction was confirmed. Carbon dioxide is not gluconeogenic in the usual sense of causing net formation of glucose or glycogen even though it leaves its trace in the form of isotopic label. The diminution of labeling from 1-^{11}C-lactic acid compared to 2,3-^{11}C-lactic acid in glycogen results from the fact that one carbon of the six in glucose is diluted by half with unlabeled CO_2 in the roundabout route through part of the citric acid cycle that the three-carbon precursors must take to get into the gluconeogenic pathway. This result made clear the power of isotopic labeling experiments and at the same time underscored the caution necessary in interpreting the results. As a junior member of this research team, Jack took note of these lessons, which he would have occasion to revisit in his later research.

Jack's individual project in the Hastings group was to explore glycogen synthesis in an in vitro system using rat or rabbit liver slices. He profusely credits Birgit Vennesland, a postdoctoral fellow in Hastings's group, with serving as his day-to-day lab mentor, guiding his development as a bench scientist and instructing him in the art of research. A key to success in the liver slice system was their discovery, in collaboration with Frances Nesbett, that an ionic milieu containing potassium, magnesium, and calcium ions—more closely resembling the intracellular environment than simple Ringer's solution—greatly enhanced glycogen synthesis from glucose. The labeling of one carbon in six of glucose by $^{11}CO_2$

was observed during gluconeogenesis in vitro from pyruvate, as predicted from their previous results in vivo.

After the country entered fully into World War II in December 1941, members of the Harvard collaboration headed in diverse directions. Hastings remained as chair of biological chemistry at the medical school but was often called to Washington for wartime administrative duties. Arthur Solomon departed with his precious Geiger counter. Turning to a question that had struck him as interesting from his biochemistry course at Michigan, Jack rounded out his dissertation research with a look at fatty acids as possible precursors of glycogen, using 1-^{11}C-labeled acetic, propionic, and butyric acids. Acetate and other even-numbered carbon-chain fatty acids were long known, from nutritional experiments in animals, not to be glucogenic. In view of how the Krebs citric acid cycle works, that is understandable because two carbons (as acetate or acetyl CoA) are eliminated as CO_2 before the key gluconeogenic intermediate, oxaloacetate, is encountered. Thus, no net carbon mass can enter glycogen from acetic acid, there being no alternative pathway from this precursor to 3- or 4-carbon intermediates in animals. One would predict from the details of the Krebs cycle, however, that radioisotopic atoms from acetate should end up in carbohydrates. In nearly the last metabolic experiments employing ^{11}C from the Harvard cyclotron, and with a crude electrometer as detection device, Jack tested the 1-^{11}C-labeled fatty acids. 1-^{11}C-propionic acid was converted in the liver slices to labeled glycogen, a not unexpected result since propionic acid is itself gluconeogenic. His results were ambiguous with the even-numbered acetic and butyric acids and he was thus frustrated in confirming the expected isotopic incorporation from these precursors. Nevertheless, given the difficulties of working with ^{11}C, in addition to the fact that priorities were shifting in other directions, he

had taken these studies as far as he could. He completed his dissertation research in January 1943 and spent several months working with Eric Ball in the Biological Chemistry Department on a war-related project on mustard gases before taking up his first independent academic appointment in the fall of that year.

FACULTY APPOINTMENT AT PENNSYLVANIA

Through Baird Hastings and Eric Ball, Jack learned of an instructorship in the Department of Physiological Chemistry at the University of Pennsylvania, where D. Wright Wilson was chair. He successfully applied for an appointment in a department he described as "deceptively sleepy" in comparison with the energetic environment at Harvard. A notable adjustment was the necessary switch from the frantic pace demanded for work with short-lived ^{11}C to the more leisurely one allowed in tracer experiments employing the stable isotopes ^{13}C and ^{15}N. In Philadelphia he had access to a mass spectrometer for analyzing stable isotopes, but not (at that time) a source of radioactive carbon. The description "sleepy" soon proved to be a deceptive one.

Jack joined an established research department consisting of Wilson, Samuel Gurin, and Warwick Sakami (a new Ph.D.) in studying the metabolic fate of acetoacetate in homogenates of guinea pig kidney. The literature on acetoacetate metabolism using nonisotopic methods offered conflicting results on whether this substance could be converted to citrate. Results of Krebs and Eggleston and of Weil-Malherbe showed that acetoacetate was largely reduced to ß-hydroxybutyrate, at least in heart preparations. In the guinea pig kidney system Jack found that α-ketoglutarate enhanced the utilization of acetoacetate but not through increased formation of ß-hydroxybutyrate. When 1-^{13}C-acetoacetate was incubated, 5-^{13}C-α-ketoglutarate was isolated. The labeled carbon atom,

as would also be the expected result starting with acetate labeled in position 1, would thereafter lead to labeling of both carboxyl groups of fumarate and oxaloacetate, and thence the three-carbon precursors of glucose and glycogen. The result of this experiment with 1-^{13}C-acetoacetate in a way bore on the unsuccessful attempt Jack had made in his dissertation research concerning 1-^{11}C-acetate as a potential isotopic precursor of glycogen, even though neither of these even-carbon ketogenic compounds was glucogenic.

At the time, two proposals for fatty acid oxidation to ketone bodies, such as acetoacetate, were in contention: one, favored by W. C. Stadie, was that long-chain fatty acids were first oxidized at alternating positions to give polycarbonyl fatty acids that were subsequently split to the four-carbon acetoacetate. The second idea, promoted by Sidney Weinhouse, posited that fatty acids underwent sequential ß-oxidation followed by cleavage to the two-carbon compound, acetate, two of which combined to form acetoacetate. Using rat liver slices, the Weinhouse group found that 1-^{13}C-octanoate was converted to acetoacetate in which the 1- and 3-positions were both labeled, a result supporting his proposal that acetate was a necessary precursor in the formation of ketone bodies via acetoacetate. One loose end remaining, however, was the possibility that octanoate was first oxidized to acetoacetate, which then was split to two-carbon compounds that subsequently recombined. Using 1- and 3-labeled acetoacetate, Sakami, Buchanan, and Gurin found that after incubating with rat liver slices, these two compounds could be re-isolated and shown to retain the initial labeling pattern without any scrambling. Taken together with Weinhouse's observations these findings seemed to nail down the Weinhouse scheme concerning the origin of ketone bodies from fatty acids; that is, a two-carbon intermediate, presumably acetate or its metabolic equivalent, is the necessary intermediate. One

more significant result on this project emerged before Jack moved on to other fields: they repeated the exposure of rat liver slices to 1-^{13}C-octanoate, followed by isolation of acetoacetate. Decarboxylation of the acetoacetate showed that both the 1- and 3-positions contained label, as the Weinhouse group had found, but that the 1-position (carboxyl) was more heavily labeled than the 3-position (carbonyl). A simple coupling of two identical acetate moieties could not explain such unequal labeling; the resolution of this question remained open for several years until Wakil demonstrated the importance of malonyl CoA, formed by ATP-dependent carboxylation of acetyl CoA, in coupling acetyl units to form extended fatty acid chains.

We have reviewed in some detail these early experiments in which Jack had a hand both to show his contributions to the growing knowledge about these central pathways of metabolism and to trace the evolution in his understanding of how complex metabolic systems may be studied and interpreted. These were indeed necessary transitional steps in moving the targets of investigation from the whole animal to isolated enzyme systems. Not only were the questions being asked quite basic ones, by later standards, but the tools available to crack open the problems were also primitive compared with what would be available in any biochemistry laboratory 10 to 15 years later.

A NEW DIRECTION: PURINE BIOSYNTHESIS

It was the time in Jack's career for him to establish a new and independent research program. As a consequence of the interface of his earlier work with the Krebs citric acid cycle, he had studied all of Hans Krebs's papers, including those dealing with urea synthesis, amino acid metabolism, and purine synthesis. Jack recognized that Krebs's great contribution to biochemistry was in calling attention not

solely to individual enzymatic steps but also primarily to complex metabolic interrelationships; that is, to metabolic pathways. Purine biosynthesis seemed ripe at that moment for such an approach. A serendipitous encounter with one of Jack's first research students may have been the specific impetus for the purine project, at least in the recollection of that student. John Sonne was, in 1945, a second-year medical student at the University of Pennsylvania Medical School looking for something more stimulating to do than just to take preclinical courses. He approached Jack about working in his lab and as he described their first meeting in a letter written on the occasion of Jack's 70th birthday, Sonne "had, for some reason, been interested in gout. Jack and I talked, and out of this came a project for working on uric acid synthesis." Gout, significantly, was a major topic discussed in Sonne's first-year biochemistry course. Thus, the inspiration for the purine project may well have been a combination of Krebs's genius and the excitement a beginning medical student gained from his studies. In any case a project was commenced that was quick to yield important results.

Krebs and coworkers had opened the study of purine synthesis several years earlier with their finding that hypoxanthine was the first purine product formed in pigeon liver slices. This observation appeared to negate an older hypothesis that uric acid might be formed by condensing two molecules of urea with a three-carbon moiety. It also argued against Krebs's conjecture that purine synthesis was in some way connected to the urea cycle. His group also reviewed chemical methods gleaned from the old German chemical literature for the systematic degradation of uric acid to component atomic and molecular parts. Since tracing the metabolic precursors of the various positions of uric acid requires several chemical degradation procedures, the

requirement for a ready source of the material in substantial quantity precluded solving this problem using tissue slices. For that reason John Sonne and Jack initiated their isotopic tracer work in vivo using pigeons. These are ideal objects of study; being uricotelic they excrete lots of uric acid as the end product of nitrogen metabolism. Uric acid can be isolated easily in crystalline form from pigeon excreta, the substance, Jack did not hesitate to acknowledge, on which his career was founded. Pigeons could be obtained either from a commercial source (which Ed Korn recalls was a farmer who collected them from his barn at night) or, more surreptitiously, by members of the research group in Rittenhouse Square in Philadelphia.

It occurred to Jack and John that the three-carbon backbone of uric acid might derive from lactic acid, and so they fed pigeons 1-^{13}C- and 2,3-^{13}C-lactic acid, synthesized by their colleague, Adelaide Delluva. The excreted uric acid was subjected to chemical degradation to separate the various atomic components. The only clear result was that when 1-^{13}C-lactic acid was administered, C-6 of uric acid was labeled with about the same isotopic content as respired CO_2. As confirmation, when ^{13}C-bicarbonate was fed to the animals, again only C-6 contained significant label, and with the same isotope content as respiratory CO_2. Evidently C-2 and C-8 were derived from a different precursor, and the hypothesis of lactate being a direct precursor was disproved. Since C-6 appears to come directly from CO_2, glycine and acetic acid were considered as possible two-carbon donors of C-4 and C-5 in uric acid. Sam Gurin supplied 1-^{13}C-acetic acid, and this was fed. Isotopic label appeared, unexpectedly, not in C-4 or C-5 but in C-2 and C-8 from this source. It soon turned out that the observed labeling arose from a contaminant in the acetic acid preparation. Recalling Krebs's result that hypoxanthine was the first purine formed before

uric acid, Jack reasoned that one or both rings of uric acid might be closed in a condensation with a carbon compound at a lower oxidation state than CO_2, namely, formic acid as in the chemical formation of benzimidazole from *o*-phenylenediamine. ^{13}C-Formic acid was therefore prepared and immediately found to label both C-2 and C-8 in uric acid. This was a signal discovery in what would be called "one-carbon metabolism," centered on the participation of folic acid derivatives, and one that would play prominently in Jack's future research.

To test the other potential two-carbon precursor, 1-^{13}C-glycine was prepared and found to contribute isotope to C-4 of uric acid. While they did not have ^{15}N-labeled glycine to test, Jack and Sonne observed that a large pool of unlabeled glycine diluted the amount of isotopic nitrogen that appeared in N-7 and N-9 when pigeons were fed ^{15}N-labeled ammonium salts. This isotope dilution was consistent with the idea that glycine entered intact into the purine ring, a conclusion subsequently supported by Shemin and Rittenberg using ^{15}N-glycine, and by Karlsson and Barker, who showed that 2-^{13}C-glycine labeled the 5-position of uric acid.

SABBATICAL IN SWEDEN

In about one year's time after embarking on this project, Jack along with Sonne and Delluva were able to identify the metabolic precursors of the positions of the purine ring save the nitrogens at positions 1, 3, and 9. These could not be resolved in whole-animal studies because of rapid interchange between ammonium salts and amino acids, such as aspartate, glutamate, and glutamine. Clearly the problem had to move from the whole animal to in vitro systems focusing on the separate enzymatic steps in the process. At Baird Hastings's timely suggestion Jack agreed that this would be a good time to take a sabbatical and gain experience with enzymes

and proteins. He was awarded a National Research Council Fellowship, and beginning in November 1946 he entered a fruitful two-year stay in the laboratory of Hugo Theorell at the Karolinska Institutet in Stockholm, Sweden.

While Jack found the quarters at the institute ancient, the scientific atmosphere was of the highest order, owing to the presence of a group of distinguished senior members and an outstanding class of younger postdocs from Europe and the United States. The Swedish faculty consisted of Einar Hammarsten, Hugo Theorell, Torbjörn Casperson, J. Erik Jorpes, and Sune Bergström. Visiting scientists in Theorell's lab, in addition to Jack, included Richard Abrams, Britton Chance, Ralph Holman, Christian Anfinsen, Christian de Duve, Andreas Maehley, and Elèmer Mihalyi. Peter Reichard, Karl Gustav Paul, Sven Paléus, and Bo Sorbo were graduate students. Four from this outstanding group at the institute would eventually become Nobel laureates.

Jack and Chris Anfinsen (who coincidently had been roommates for a year while they were graduate students at Harvard Medical) set to work to purify aconitase from pig heart. This was not the best choice, since the enzyme was difficult to assay and quite unstable during purification. Standard methods at the time for purifying enzymes relied on differential precipitation with salts or organic solvents and in some cases electrophoresis: the more powerful techniques of fractionation by ion exchange chromatography and gel filtration were yet to be devised. Although his accomplishments in the lab were admittedly rather disappointing to Jack, his social life in Sweden thrived. Not only did he master the Swedish traditional dance, the hambo, he also enjoyed the long summer nights swimming and sailing in the archipelago. Most important of all, he met Elsa Nilsby, who would soon become his wife, his companion, and soulmate for the rest of his life.

Returning to the University of Pennsylvania in 1948, Jack energetically restarted his purine synthesis program. John Sonne rejoined Jack, now as a postdoctoral fellow, and the group in time became a vibrant team of graduate students and postdocs—I Lin, Martin Schulman, William Williams, Edward Korn, Frixos Charalampous, Hans Brandenberger, Charles Remy, Joel Flaks, Bruce Levenberg, and Lewis Lukens among them. In the intervening two years G. Robert Greenberg at Western Reserve University began his study of purine biosynthesis. Among his important observations was that hypoxanthine is formed in cell-free homogenates of pigeon liver when ^{14}C-labeled formate and bicarbonate were used as tracers.[1,2] Greenberg also discovered that the nucleotide inosinic acid (9-(5'-phosphoribosy)-hypoxanthine) was initially formed in this system as a precursor of hypoxanthine. It was presently found in both labs that the soluble protein fraction of homogenates supported formation of inosinic acid, a result that transformed the problem into one of enzyme fractionation and characterization of intermediates.

Jack's group examined the route by which the ribonucleotide, inosinic acid, was formed from hypoxanthine. One possibility was coupling of the base with ribose-1-phosphate by the enzyme, nucleoside phosphorylase (previously identified by Herman Kalckar) followed by phosphorylation of the resulting inosine. Alternatively, the base might be directly condensed with a phosphoribosyl donor to yield inosinic acid in one step. Ed Korn, a student of Jack's, showed the latter to be the case: the pathway did not proceed through the nucleoside inosine. Two separate ethanol-precipitated fractions of pigeon liver extract were required, one to produce an active phosphorylated form of ribose-5-phosphate and the second containing a phosphoribosyl transferase to condense the intermediate to hypoxanthine. One fraction, studied by Charalampous and by Remy, produced a new ribose phosphate

compound from ribose-5-phosphate and ATP, although its instability made characterization difficult. Ribose-1,5-diphosphate was considered as a possibility, but Arthur Kornberg's group, which had been studying the related reaction from adenine to adenosine 5'-phosphate, definitively identified the active intermediate as 5-phosphoribosyl-1-pyrophosphate (PRPP). Two related phosphoribosyl transferases were soon purified by the Buchanan and Kornberg groups: one specific for hypoxanthine, guanine, and certain non-natural purines, such as 6-mercaptopurine, and the second reactive with adenine and 5-amino-4-imidazolecarboxamide. It would soon be found that PRPP was the active agent in adding the 5-phosphoribosyl group during assembly of the purine ring, and that it entered the pathway at a very early stage. The ribophosphorylation of the purine bases to their nucleotide forms is thus not part of the *de novo* pathway but rather are salvage reactions.[3]

MOVE TO MIT AND CULMINATION OF THE PURINE BIOSYNTHESIS PROJECT

At about this time Jack was being eyed by MIT as a possible new faculty member, one who would be able to nucleate development of a vigorous biochemistry program in the Department of Biology whose strength had largely been in biophysics. This high-level recruiting effort involved both the chair of biology, Francis O. Schmitt, and the president of the institute, James Killian. Jack had risen to the rank of full professor at Penn, and he would move as a full professor with promise of a large, fully equipped laboratory in the new Dorrance biological sciences building. He accepted the offer and in 1953 was appointed head of a newly established, semi-independent Division of Biochemistry in the Department of Biology, bringing with him his continuing graduate students, Joel Flaks, Bruce Levenberg, and Lew Lukens, and his postdoc, Chuck Remy. Within a year or two he had attracted several

new students, including postdocs Leonard Warren, Armand Guarino, Samuel Love, and Fredrick Hatch, and graduate students Standish Hartman, Richard Miller, Irving Melnick, Thayer French, and Allan Larrabee. He was also an immediate contributor to departmental development, his first faculty hire being Gene Brown in 1954. Shortly thereafter he was instrumental in bringing Salvador Luria, Phillips Robbins, Lisa Steiner, Paul Schimmel, and Vernon Ingram to the department. These new recruits, to which many others were later added, formed the basis for an entirely new direction of growth in biology at MIT, which led in time to its being identified as one of the strongest in the country, a position it has held ever since. President Killian, in his autobiography many years later, observed that bringing Jack to MIT was among the most important recruitments during his tenure, as it directly led to elevating the biochemistry program to the top ranks in the field.

Continuing the purine work in the MIT lab now with its own isotope-ratio mass spectrometer, the group was able to assign unambiguously the precursors of the nitrogen positions of the ring, using ^{15}N-labeled metabolites with an ethanol fraction of pigeon liver. In addition to N-7, derived from glycine as was already known, N-3 and N-9 were found to arise from the γ-amide nitrogen of glutamine and the N-1 position from aspartate. This work completed the identification of the metabolic precursors of all positions of the purine ring, the results of which are summarized in Figure 1 showing the original target, uric acid.

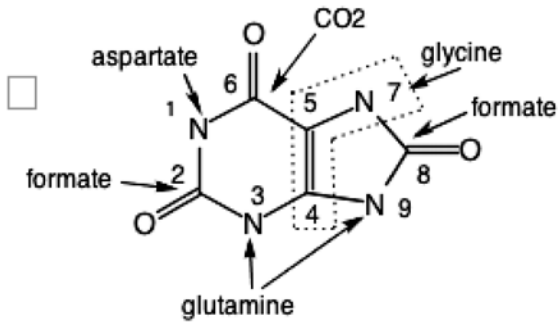

FIGURE 1

The biosynthetic pathway was attacked from both ends. Both Greenberg's and Buchanan's groups were able to identify two early intermediates that were labeled with ^{14}C-glycine or ^{14}C-formate: 5-phosphoribosylglycinamide (GAR) and 5-phosphoribosyl-N-formylglycinamide ribonucleotide (FGAR). Thus, it was clear that phosphoribosylated intermediates participated at the earliest stages in assembling the purine ring system. Greenberg's group soon showed that formation of GAR occurred in two steps: amino group transfer from glutamine to PRPP, forming 5-phosphoribosylamine, followed by ATP-dependent coupling of glycine with this product.

Working out the several middle steps of the pathway was greatly facilitated by a trick that allowed accumulation of large amounts of FGAR for use as a substrate in subsequent steps. The glutamine analog, O-diazoacetyl-L-serine (azaserine) was found by Levenberg and Melnick to inhibit irreversibly the glutamine-dependent enzyme directly following FGAR in the sequence, but not the one by which 5-phosphoribosylamine is formed (although another glutamine analogue, 6-diazo-5-oxonorleucine, blocks both enzymes). Fortuitously, most of the intermediates after FGAR are diazotizable amines that

can be coupled to N-(1-naphthyl)-ethylenediamine to give products with characteristic absorption spectra in the visible region. The several steps were unraveled by graduate students Levenberg, Lukens, Melnick, and Miller using enzyme fractionation and this assay method. Briefly, glutamine reacts next with FGAR to yield an amidine containing the presumptive N-3 of the purine ring, followed by formylation at the amino position (N-7-to-be) in the presence of a formylated derivative of tetrahydrofolic acid (subsequently found to be 10-formyltetrahydrofolate) and closure of the 5-membered ring to give 5'-phosphoribosyl-5-aminoimidazole. In the presence of bicarbonate (without ATP participation) a carboxylic group is affixed to the ring in what will become C-6 of the purine ring. An ATP-dependent reaction with L-aspartate then couples 5'-phosphoribosyl-4-carboxy-5-aminoimidazole to form a succinamide derivative and in the next step the four-carbon side chain is eliminated as fumarate with the formation of 5'-phosphoribosyl-5-amino-4-imidazole carboxamide. This key intermediate, which is two enzymatic steps removed from inosinic acid, had meanwhile been under study in the reverse direction by Flaks and Warren. In the presence of tetrahydrofolic acid, acting as a formyl group acceptor, the six-member ring of inosinic acid opens to form the same substance; in the forward direction this reaction constitutes the final step in the purine *de novo* biosynthetic pathway. The pathway is summarized in Figure 2.

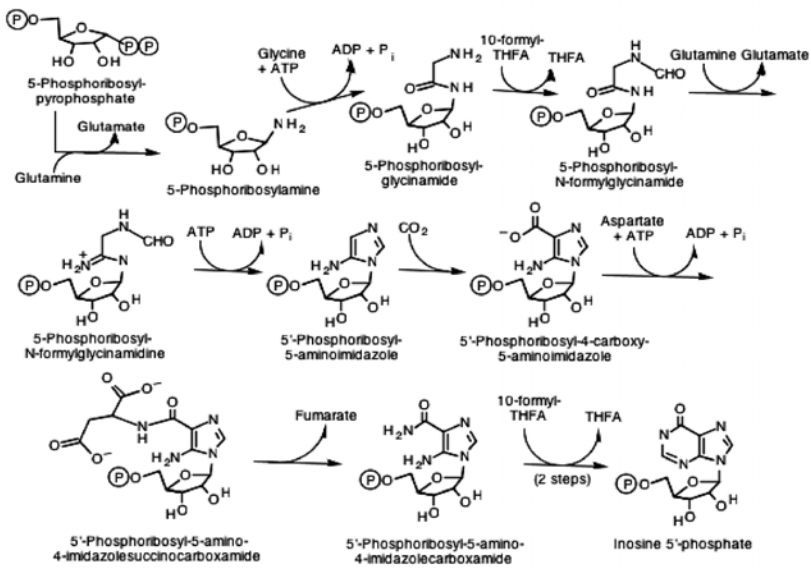

FIGURE 2: Pathway of biosynthesis *de novo* of inosine 5´-phosphate.

The mapping out of the *de novo* pathway was essentially completed by 1956. Jack oversaw and coordinated the project mainly with his mixed crew of experienced and beginning graduate students. His style, reflecting his own development as a bench scientist, was to assign his students their own projects with clear boundaries to minimize territorial disputes. New graduate students were first placed alongside more experienced ones to teach them the ropes and to help socialize them into the group. When the research is going well, as it was for nearly all participants at the time, lab spirit is high, a cooperative atmosphere develops, and everyone is happily productive. The conjunction of these salubrious forces in a research group is rare, and it may in part be good fortune. Nevertheless, without doubt Jack deserves great credit for managing his large group and mentoring them through their early scientific careers, as even his faculty colleagues noted with admiration. He especially is remembered for his

patience in teaching his students the art of writing up their work for publication: this was the point at which most of his students learned how to construct sentences, paragraphs, and papers.

The purine project spun off many others. Conversion of inosinic acid to guanylic and adenylic compounds was pursued in other laboratories. In Jack's group it was the branch point from which several other investigations grew. Among these was the attempt to identify the site of covalent binding of azaserine to FGAR amidotransferase. This was of interest not only as it was the first example of a naturally occurring, active-site-directed irreversible inhibitor but also as it could shed light on the mechanism of the reaction. Graduate students Thayer French and Kiyoshi Mizobuchi, along with postdocs Igor Dawid and Richard Day, started the problem using the purified enzyme from liver and later from Salmonella. The project turned out to be more difficult than expected, eventually adding Shiro Ohnoki, Bor-Shyue Hong, Duane Schroeder, Joyce Allison, George Kenyon, Jean-Marie Frère, and Heng-Chun Li to the team. With persistence an active-site cysteine was determined to be the point of attachment. These workers pursued detailed mechanistic and structural studies on the enzyme, concluding that the thiol group of this cysteine served as nucleophile in displacing the NH_2 group from glutamine during the transferase reaction.

METHIONINE BIOSYNTHESIS

As the purine biosynthesis project was winding down Jack became intrigued by reports in the literature about possible connections between methionine, vitamin B_{12}, pernicious anemia, purines, and DNA synthesis. The nutritional interrelationship between folic acid and B_{12} had been well established in bacteria as well as in animals. Another obvious link was through the role of folate derivatives in purine and

methionine formation. He put the problem to a group of his students, consisting initially of postdoc Fred Hatch and graduate students Al Larrabee, Renata Cathou, and Shige Takayama. Two methionine-requiring mutants of *Escherichia coli* were obtained from Bernard Davis, one of which evidently was defective in B_{12} synthesis since its nutritional need for methionine could be met by this vitamin. Jack's group found that extracts of the two strains complemented each other in forming methionine in vitro. Upon purification one strain (205-2) yielded a functional B_{12}-containing enzyme but otherwise lacked an activity needed to form methionine. Purified extracts of the second strain (113-3, grown with B12), when incubated with 5,10-methylenetetrahydrofolic acid and the reducing agent, $FADH_2$, produced a new, reduced-folate intermediate. The evidence suggested that this was 5-methyltetrahydrofolate, a conclusion corroborated by Warwick Sakami, who synthesized the same material by chemical reduction of 5,10-methylene tetrahydrofolate. In the presence of catalytic amounts of ATP the extract from 113-3 was shown to effect transfer of the methyl group from 5-methyltetrahydrofolate to homocysteine, forming methionine.

Jack's methionine group, which grew to include Sumner Rosenthal, Richard Loughlin, Howard Elford, Louis Smith, and Barbara McDougall, looked further into the mechanism of methyl group transfer from 5-methyltetrahydrofolate to homocysteine and thence to other methyl receptors. His group and the work of others concluded that in the process

homocysteine + 5-methyltetrahydrofolate → methionine + tetrahydrofolate

the methyl group is carried as a metallo-cobalt ligand of the B_{12}-containing methyltransferase, but that S-adenosylmethionine also plays an obligatory catalytic role (hence the requirement for ATP in the earlier experiments). An important concept, that of the methyltetrahydrofolate trap emerged

from the studies by the Buchanan group, one that clearly links the metabolism of purines and methionine to folate and B_{12} deficiencies. Tetrahydrofolate is necessary as an acceptor of one-carbon (formyl or methylene) groups arising from, for example, the conversion of serine to glycine, after which oxidation to the formyl group provides the precursor of C-2 and C-8 of the purine ring, as discussed above. A competing process, reduction of a form of formyltetrahydrofolate to 5-methyltetrahydrofolate, is effectively an irreversible reaction. Thus, if the latter compound cannot be recycled by transfer of its methyl group to homocysteine, as would be the case in B_{12} deficiency, the tetrahydrofolate cycle is blocked, resulting in accumulation of nearly all folate coenzymes in the cell as the trapped 5-methyl form. Pernicious anemia is one clinical manifestation of a B_{12} deficiency state; the methyltetrahydrofolate trap hypothesis accounts for the interference with DNA synthesis in pernicious anemia through the effect on purine (and thymine) biosynthesis.

BACTERIOPHAGE ENZYMOLOGY AND REGULATION

In the 1950s and 1960s bacteriophage emerged as one of the principal new model systems for the study of DNA replication and protein synthesis. Jack's interest in this field was piqued by a paper published by G. R. Wyatt and Seymour Cohen at Penn, who had shown that the DNA synthesized in bacteria under the direction of T-even coliphages contained 5-hydroxymethylcytosine in place of cytosine. His interest in this research may have been further stimulated by the decision of his graduate student, Joel Flaks, to go to Cohen's laboratory for his postdoctoral studies. At the time, Cold Spring Harbor Laboratories, a center for bacterial virus studies, ran a well-known summer course in bacteriophage initiated by Max Delbruck in the late 1940s. Jack decided to go with Flaks to take that course in the summer of 1956. It was there

that Jack began a friendship with Salvador Luria that later led to Luria's recruitment to the Division of Biochemistry at MIT.

The unique occurrence of hydroxymethylcytosine in T-even bacteriophage DNA seemed likely to be a key to understanding how the bacteriophage redirects synthesis from the host's DNA to its own. An important enzymological clue came from the discovery by Flaks and Cohen in 1957 that a phage-induced enzyme in infected *E. coli* cells, dCMP hydroxymethylase, directly transfers the hydroxymethyl group from 5,10-methylenetetrahydrofolate to dCMP, forming hydroxymethyl dCMP (dHMP). In 1956 Kornberg, Lehman, and Simms reported much reduced rates of DNA synthesis by partially purified DNA polymerase (now known as DNA polymerase I) isolated from phage-infected cells as compared with rates in uninfected cells. In Jack's lab James Koerner and Marilyn Smith (1960) were able to identify and purify a phage-encoded protein that accounted for the suppressed bacterial DNA synthesis: deoxycytosine triphosphatase (dCTPase), which hydrolyzes dCTP and dCDP to dCMP. Following reports of Sinsheimer and of Volkin that the HMC in phage was partly in glucosylated form, Koerner and S. Varadarajan synthesized glucosylated dHTP and found that this deoxynucleoside triphosphate was incorporated into DNA in phage-infected *E. coli* cells but not in uninfected cells, a result indicating a difference in the substrate specificity of a presumed phage-induced and bacterial DNA polymerase. These findings provided a mechanistic explanation for the suppression of bacterial DNA synthesis in phage-infected bacteria and began an interest in bacteriophage that continued for more than 20 years. Along the way the Buchanan lab produced several discoveries that had major implications for the general topics of DNA synthesis and the regulation of protein synthesis.

Progress in phage genetics was given a jump-start in 1959 with the publication by Robert Edgar and associates of the isolation of a series of amber mutants that contained misplaced stop codons, causing premature arrest of protein translation. Luria obtained these mutant phages in advance of their publication and with Luria, John Wiberg, and Marie-Luise Dirksen, Jack began to ascribe biochemical functions to the individual genes—a major advance in the field. The first to be identified (1962) was gene 42, which Jack's team found encoded dCMP hydroxymethylase. The Buchanan group further showed that mutations in phage gene 42 caused changes in enzyme activity and stability. These experiments offered confirmation to the seminal work of Hershey and Chase showing that phage-induced proteins were specifically encoded by phage genes.

In the early 1960s it was becoming apparent among phage researchers that protein synthesis was temporally regulated after infection of bacteria. The terms "early" and "late" were used to designate proteins that appeared prior or subsequent to DNA replication, a process that began approximately 8-10 minutes after infection. One would suspect that the proteins necessary for suppression of bacterial DNA and replication of phage DNA would be among the early proteins. This was indeed the case. These proteins did not appear until about three minutes postinfection, and their synthesis stopped as DNA synthesis began. Late proteins did not appear until the early protein synthesis had ceased. It is interesting that experiments on the kinetics of phage protein synthesis involved experimental points taken at minute or shorter intervals. This would not have disturbed Jack since he was used to radioisotope experiments that needed to be conducted with the utmost rapidity.

The effect of the onset of DNA synthesis as a negative regulator of early phage protein synthesis was further studied

in experiments in which Dirksen, Wiberg, Koerner, and Buchanan blocked phage DNA synthesis by irradiating the bacteriophage before infection. In this elegant experiment they observed that prevention of phage DNA synthesis allowed the continued synthesis of early proteins long after the 10 minute time when they normally ceased their synthesis. This confirmed the hypothesis that the cessation of DNA protein synthesis after 10 minutes postinfection was directly dependent on the time of first DNA synthesis. A similar conclusion was reached using *amber* mutants that attenuated DNA synthesis.

This work stimulated a general interest in the regulation of phage protein synthesis. A major question was whether the temporal regulation of phage protein synthesis could be correlated directly with the amount of mRNA for those proteins, or was additionally regulated by post-transcriptional mechanisms. This issue was first addressed with Robert Grasso using DNA-RNA competition-hybridization, as described in a paper published in *Nature* in 1969. These experiments showed that inhibition of protein synthesis with various inhibitors led to the up-regulation of several classes of mRNA and led to the categorization of certain mRNA species as "immediate early" and "delayed early," depending on their time of appearance. More precise measurements of the kinetics of production of specific mRNA species came from experiments conducted with George Guthrie, Akira Kuninaka, and Kenneth Lembach in which transcription was allowed to occur in phage-infected *E. coli* protoplasts in the presence or absence of protein synthesis, and then further RNA synthesis was inhibited and translation was allowed to occur. They found that the transcription inhibitor, rifampicin, worked best in these experiments because its activity was rapid and complete. These experiments clearly demonstrated the kinetics of both message accumulation and protein synthesis and allowed for

the relationship between the two to be studied with much greater precision. An important conclusion of this work was that transcription of specific classes of mRNAs subsequent to phage infection was differentially regulated by the synthesis of phage-induced proteins.

The studies on regulation of protein synthesis then moved on to fully in vitro systems, as first described by Salser, Gesteland, and Bolle in the late 1960s. The first of these studies in Jack's lab, published with Shigeru Sakiyama in 1971, involved isolation of RNA at different times from phage-infected cells. The purified RNA was then incubated with bacterial lysates. In this paper the kinetics of production of deoxynucleotide kinase mRNA was measured using the in vitro system as an indication of the temporal production of deoxynucleotide kinase mRNA under different conditions. This pattern was then compared with the profile of deoxynucleotide kinase production in intact phage-infected bacteria. Using this system, they were able to determine the kinetics of deoxynucleotide kinase mRNA formation, to classify it as a Class II, or delayed early message, and to compute the half-life of the message to be 4.5 minutes. This work was confirmed and extended over the next several years by Peter Natale and Carrie Ireland and also by Paul Cohen, who came from Rhode Island to spend a year's sabbatical with Jack at MIT in 1973.

An important theme that emerges from the work on mRNA and protein regulation in bacteriophage was Jack's ability to recognize novel patterns in his data that did not conform to then current dogma. At this time the conventional wisdom was that transcription and translation were closely linked in prokaryotes and that when mRNA was synthesized, it was rapidly and completely translated. Jack realized that his own data contradicted this viewpoint and he was unafraid to discuss the possibility. In the discussion of the 1970 paper

with Lembach they state: "One might suggest that translational mechanisms come into play to regulate the utilization of the messengers for dCTPase and dCMP hydroxymethylase in these cells…one might postulate the formation of a specific factor which regulates the translation of mRNA for these enzymes in the infected cell." The following year, in the paper on deoxynucleotide kinase mRNA with Sakiyama, they further state: "If the synthesis of deoxynucleotide kinase *in vivo* were completely under transcriptional control, one could expect that the amount of functional deoxynucleotide kinase messenger might be proportional to the amount of enzyme synthesis *in vivo*…This is not the case in our experiments." Clearly, Jack was conscious that his experiments revealed a new type of control of protein synthesis in bacteria that had not been previously observed on the individual protein level. Such control of phage protein synthesis was later shown to exist and to be in some cases dependent on the *regA* gene product, which encoded a translational repressor protein.

A STEP INTO CELL BIOLOGY: MITOGENESIS

In 1964 Jack felt confident enough with the bacteriophage studies to want to go and learn something new. He embarked on a year's sabbatical in the laboratory of Renato Dulbecco at the relatively new Salk Institute. He was assigned to search for a factor in mouse salivary glands that could stimulate the growth of hepatocytes in vitro. Although this particular endeavor was not successful it did introduce him to the exciting field of growth control in mammalian cells. Later after returning to MIT, Jack's lab took up the question of how cell growth was regulated in cultured eukaryotic cells.

At the time, serum was the growth stimulus of choice and the nature of the factor(s) in serum that stimulated cell growth was being pursued in several laboratories. A report from Bart Sefton and Harry Rubin appeared in 1970 that

showed the protease trypsin could stimulate the growth of cultured mammalian cells. This topic appealed to Jack because it involved the activity of an enzyme, which was a phenomenon he understood well. A talented graduate student, Lan Bo Chen, took this on as his dissertation topic, and the lab produced a noteworthy series of papers on the subject. They focused on the activity of the coagulation protease thrombin in part because it was very specific in its cleavage of particular amino acids and in part because there was a thrombin expert in the department in David Waugh. Chen and Buchanan first showed that thrombin was indeed a mitogen. They, along with Nelson Teng, Tung-Tien Sun, and Bruce Zetter, set out to determine the nature of the proteins that were cleaved from the cell surface by thrombin. The first protein identified was the large external transformation-sensitive protein identified by Richard Hynes at MIT and Erkki Ruoslahti. Hynes originally called this protein LETS, but the name fibronectin eventually became the accepted nomenclature. Additional proteins cleaved by thrombin were also identified. Over time, however, it became apparent that the proteolytic activity of these proteases was only one contributor to their mitogenic activity. Receptors binding these proteins were also present on the cell surface and initiated signaling events that mediated the mitogenic response.

The final part of Jack's work on eukaryotic cells dealt with mechanisms of transformation by RNA viruses, such as the Rous sarcoma virus. He felt that the expertise they had gained by studying the synthesis of specific phage proteins could be brought to bear on the question of the proteins involved in the process of viral transformation of eukaryotic cells. The work was initiated by James Kamine, who brought his own expertise on these viruses to the lab. By 1977 they, along with two other labs, had successfully identified distinct proteins that were implicated in the transformation process.

The best characterized of these proteins was, of course, the *src* gene, which was originally identified by Erikson and colleagues, who then showed that the gene product was a tyrosine kinase. Questions regarding the nature of the molecules that were phosphorylated by kinases, such as *src*, occupied the laboratory until it closed. The last paper in Jack's curriculum vitae, by Laudano and Buchanan (1986), describes the biochemical analysis of the phosphorylation of specific tyrosines in the viral *src* protein by its endogenous kinase activity. It is safe to say that the study of enzymes was Jack's first and foremost scientific love and that the desire to understand them more completely occupied the entirety of his scientific career.

EPILOGUE

Looking back at the scope of Jack's career, one must be impressed by his adaptability and fearlessness in moving boldly time and again into new areas of research. There is a central logic in how his work evolved as biology itself evolved during his active career. Jack embodied the highest standards of scientific integrity; he demanded thorough experimental justification of his students' data and rigorous analysis of their conclusions. He passed these qualities on to his students as essential parts of the scientific inheritance they derived from their beloved mentor. No less important than in their scientific education, his students grew in their personal development from his boundless support and friendship, as did the many friends and colleagues that he touched throughout his life.

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NOTES

1. By this time ^{14}C had supplanted ^{11}C and ^{13}C as the carbon isotope of choice for nearly all biochemical tracer studies, its ideal properties greatly accelerating the analysis of both in vivo and in vitro systems.

2. The gracious personalities of both Bob Greenberg and Jack, as well as their scientific abilities, fostered a friendly relationship and a good-natured competition over the years that they were pursuing the same goal. Jack was always fair and generous in giving his competitor credit where credit was due, which was often.

3. The salvage reactions, especially the one for the hypoxanthine-guanine pair nonetheless have important metabolic functions: a genetic deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPT) is the cause of the seriously debilitating condition in humans known as Lesch-Nyhan syndrome.

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