Reflections
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Metabolism in the Era of Molecular Biology

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Prologue

“No profit grows where there is no pleasure taken.
In brief sir, study what you most affect”
Tranio in The Taming of the Shrew

I have spent a lifetime in the area of intermediary metabolism and have seen it move from the center of biochemistry to the backwater of our science in a very short period, only to be re-discovered by a new generation of biologists. By 1970, the writing was on the wall for metabolism; it was largely considered a “mature area,” lacking excitement; molecular biology was the area of the future. A sure sign of this was that graduate students in biochemistry (always first to spot the trends) almost never selected their thesis research in metabolism. The course in intermediary metabolism that I taught was dropped from the curriculum of our graduate education program; our students were expected to learn all they needed to know about metabolism as undergraduates before they attended graduate school. After all, as a graduate student once said to me, “the great problems in metabolic research have been solved.” As one could easily predict, there is currently a shortage of scientists who truly understand metabolism and its regulation.

I subscribe to the Contrarian philosophy of investment. When a common stock is too popular with buyers, it is almost always a bad investment. It is better to find a company that has good fundamentals but is underappreciated by the market; in the long term, this is the always the best buy! I have found this to be very true for research. As others turned away from metabolism and toward genetics, the field remains a ripe area for further research. As long as diseases like diabetes, obesity, and atherosclerosis remain to be cured, there will be no shortage of interest in metabolism. To support this contention, I have counted five cover articles in Time magazine over the past year (2003–2004) on obesity, diabetes, and the Atkins Diet. Obviously, the general public in the United States still finds metabolism interesting! However, the problem in 1970, when I began my foray into molecular genetics, was how to enter into this virtually foreign field and still be productive enough to sustain my career.

For me personally, the transition from metabolism to molecular biology was a risky one. By planting my research in two fields, I could easily have become a “jack of all trades and master of none.” However, I had no choice; it was adapt or perish. My epiphany in this matter occurred in 1971 at a meeting on gluconeogenesis held in Gottingen, Germany. I had been invited to attend because John Ballard and I had mistakenly claimed in a paper on adipose tissue metabolism that pyruvate carboxylase was present in the cytosol (1); the intracellular location of this enzyme was actively discussed at the meeting. Despite my inexperience (I was 35 at the time), I was invited to chair a session at the meeting and, as the chair, was asked to lead a discussion on new approaches to metabolic research. I remember being rather disappointed with the answers, which ranged from developing better cell lines for metabolic studies to improving techniques for the determination of intracellular intermediates. The discussions at this meeting were published as part of the proceedings and still make interesting reading (2).
In stark contrast, at about that time molecular biologists were devising ways to isolate and characterize mammalian genes, an effort that would revolutionize biology. Metabolic research would never be the same again! I am delighted to have lived through this exciting period and to have contributed in some small way to the transition of metabolism into this new era. This Reflections is all about trying to make it doing metabolic research in the “era of molecular biology” or how I came to love the gene!

Making Early Choices

For most of us, the area we choose in graduate school is the area in which we find ourselves for the rest of our scientific lives; I chose metabolism and for better or worse have been at it ever since. Like most things in my life, this choice was not planned; it just seemed to happen. While a student at Northeastern University in Boston in the early 1950s, I worked in a small research-based company in Cambridge, Bio-Research Institute, as a co-operative work experience. There I met a young graduate of the Ph.D. program at Brown University, Tom Kelley. Tom had been a student with a member of the faculty in the Biology Department at Brown, Dr. Paul Fenton, and recommended him highly. As fate would have it, I was accepted in the graduate program in biology at Brown in 1959, and I selected Paul as my graduate advisor. For my thesis research, I studied the biochemical basis for the differences between two inbred strains of mice. One strain, the A/Fn, could be made readily obese and showed a relationship between dietary fat intake and carcass fat content. I demonstrated that during fasting these mice accumulated triglyceride in their livers. The I/Fn mice were leaner, oxidized more fatty acids to carbon dioxide, and generated more ketone bodies after an overnight fast. Adipose tissue from the A/Fn mice also had higher rates of fatty acid synthesis in vitro than did adipose tissue from the I/Fn mice (3). Isotopically labeled compounds were just beginning to be generally available for metabolic studies, and I adapted and/or developed a variety of experimental techniques to quantify metabolic flux rates in tissues from these mice and in the intact animals. Today, in the age of genomics, the genetic basis of metabolic variations in strains of inbred mice is very much in fashion. Thus, metabolic research has come full circle, but today the key technique is gene arrays to identify the underlying genetic factors that contribute to the metabolic variations leading one strain of mouse to become obese (and develop insulin resistance) whereas the other strain remains lean. The research problems never seem to change, only the techniques we use to address them! Paul Fenton was very much ahead of this time. This experience working with mice taught me a lesson of the importance of integrating metabolic information across physiological levels; from then to now, I have avoided being too much of a reductionist.

The Story of How Phosphoenolpyruvate Carboxykinase Became the Very Modern Model of a Regulated Gene

I began working on the regulation of expression of the gene for the cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK-C) in 1967; that was before those of us in metabolism thought much about genes. I had just finished 2 years as an officer in the United States Army and arrived for a stint as a postdoctoral fellow in the laboratory of Sidney Weinhouse at the Fels Research Institute, Temple University School of Medicine, in Philadelphia (Fig. 1). For me this proved to be an excellent choice.

Sidney Weinhouse had a distinguished career in biochemistry. He was among the first to use isotopes of carbon when they became available immediately after World War II. Through a series of elegant experiments using 13C-labeled octanoic acid, he proved the correctness of Knoop’s hypothesis that ketone bodies were synthesized from two carbon fragments that were generated by the β-oxidation of fatty acids in the liver (4). Weinhouse, together with David Dipietro (5) discovered hepatic glucokinase and described many of its kinetic properties as well as its induction by feeding rats a high carbohydrate diet. Sidney was also known for his work on the biochemical characterization of minimal deviation hepatic tumors (6, 7); he was the long-time Editor of Cancer Research and a respected leader in cancer biology. Sidney Weinhouse died in 2001 at the age of 94.

Sidney Weinhouse was a very busy man. As the Director of the Fels Research Institute, he had a lot on his plate. Despite being very busy, Sidney had an open door policy and he always seemed to have time to talk to me about science or about life in general. He had what I might characterize as the “if it’s not broken don’t fix it” approach to mentorship; I could do the research I wanted on a mutually agreed upon topic and if I needed advice, he was available.
Another postdoctoral fellow, John Ballard, and I began a collaboration on the development of hepatic gluconeogenesis in the rat. John was also working on the kinetic properties of galactokinase, which he purified from porcine liver (6–8), and I was involved in studies of ketone body metabolism by adipose tissue (9). I was quite familiar with the use of isotopes to determine the rate of flux of intermediates over specific metabolic pathways, and I would routinely relate the flux rate to changes in the activities of enzymes in the pathway. At about the time we began our joint research, two of the enzymes involved in hepatic gluconeogenesis, pyruvate carboxylase and the mitochondrial form of PEPCK, had been discovered at Case Western Reserve University (then Western Reserve University) in Cleveland by Bruce Keech and Merton Utter (pyruvate carboxylase) (10) and Kiyoshi Kurahashi and Merton Utter (PEPCK) (11). Later, Robert Nordlie and Henry Lardy demonstrated that there were two isoforms of PEPCK, one in the mitochondria (PEPCK-M) and one in the cytosol (PEPCK-C); pyruvate carboxylase is a mitochondrial enzyme (12). John Ballard and I developed an isotopic assay for PEPCK and pyruvate carboxylase and then used them to study the development of these two enzymes in the liver (13). In an interesting coincidence, 10 years later I became Chair of the Department of Biochemistry at Case Western Reserve University, a position also held by Merton Utter.

With our newly developed enzyme assays for pyruvate carboxylase and PEPCK, we found that there was no detectable PEPCK-C activity in the liver of rats before birth and that the enzyme appeared in a rapid fashion within the first several hours after birth, increasing to adult levels in the first day after birth (13). On the other hand, we noted that pyruvate carboxylase was present in the liver before birth and increased within the first day of life. As we understood in greater detail later, the initial transcription of the gene for PEPCK-C at birth was a key element in the initiation of hepatic gluconeogenesis that occurs in all mammals. Finally, we demonstrated, together with Helen Philippides, a Ph.D. student with John, and Linda Fisher, my first technician, that the administration of glucagon or cAMP to the rats while they were in utero would prematurely induce the appearance of PEPCK-C in the liver; insulin could block this induction. Because the induction of PEPCK-C could be inhibited by actinomycin D, we assumed that its rapid appearance at birth was due to the initiation of gene transcription. It would take another decade to definitively prove this point. In retrospect, this research formed the basis for my later venture into molecular biology.

**Glyceroneogenesis: the Metabolic Role of Pyruvate Carboxylase and PEPCK-C in Adipose Tissue**

By 1967 it had been well established that both pyruvate carboxylase and PEPCK-C were involved in hepatic and renal gluconeogenesis. So it was a real surprise that year when John
Ballard and I found pyruvate carboxylase in adipose tissue, a tissue that did not make glucose. We proposed that pyruvate carboxylase played an anaplerotic role (i.e. it replenished citric acid cycle anions) during lipogenesis in adipose tissue, because citrate efflux from the mitochondria depletes intermediates of the citric acid cycle (1). This is similar to the function of the enzyme in the liver during gluconeogenesis. We came to the totally incorrect conclusion that there were both mitochondrial and cytosolic forms of the pyruvate carboxylase in adipose tissue. It turned out that it is easy to break mitochondria during their isolation from adipose tissue and thus release the enzyme. In the same year, John and I, together with Gilbert Leveille, reported that adipose tissue also contained PEPCK-C (14). What was this gluconeogenic enzyme doing in a tissue that does not make glucose?

Several previous studies had shown that isolated epididymal fat pads could synthesize the glycerol moiety of triglyceride (glyceride-glycerol) from pyruvate in the absence of added glucose, but the authors did not speculate on how this occurred. Using specifically labeled [14C]pyruvate, we demonstrated that glyceride-glycerol was synthesized from pyruvate in adipose tissue via a pathway that involved both pyruvate carboxylase and PEPCK-C (14). Furthermore, the conversion of pyruvate to glyceride-glycerol via this pathway increased when the animal was fasted, due in part to the induction of PEPCK-C activity that occurs in adipose tissue. We proposed that this pathway (Fig. 2), which was later termed glyceroneogenesis by Eleazar Shafrir and his colleagues (15), was involved in the re-esterification of fatty acid to triglyceride in adipose tissue during fasting. Most textbooks of biochemistry indicate that 3-phosphoglycerol is synthesized in the fed state from glucose via glycolysis. Our proposed pathway of glyceroneogenesis was clearly not intuitive.

In 1967, Lea Reshef from the Department of Biochemistry, Hebrew University-Hadassah Medical School in Jerusalem, Israel visited Sidney Weinhouse at the Fels Research Institute. Lea was studying the mechanism by which ruminant animals synthesize triglyceride in the absence of dietary glucose (16, 17). It took only a brief discussion for us to realize that we were working on the same pathway and to begin a collaboration that has lasted to this day! In 1970, a collaborative paper appeared in the *Journal of Biological Chemistry* (18) in which we...
demonstrated the stoichiometry of the process of 3-phosphoglycerol formation from pyruvate for the synthesis of triglyceride in adipose tissue. Martha Vaughan (19) had reported that in isolated adipose tissue 30% of the fatty acid released by lipolysis was re-esterified to glyceride-glycerol in the absence of glucose. We confirmed this finding and proposed that glyceroneogenesis from pyruvate was the source of the 3-phosphoglycerol required for triglyceride synthesis (18).

Lea Reshef, John Ballard, and I published a number of papers on glyceroneogenesis (18, 20). However, the idea of the metabolic role of this pathway never really caught on and, until very recently, it had not been included in a text book of biochemistry; since 1970 there have been only 40 references to glyceroneogenesis in PubMed! To our delight, however, things seem to be changing. Labeling studies in mammals, including humans, have demonstrated the striking degree of fatty acid recycling that occurs, even during prolonged fasting. As an example, Jensen et al. (21) reported that humans that have fasted for 84 h recycle about 70% of the fatty acids released by lipolysis in adipose tissue back to triglyceride. This so called triglyceride/fatty acid cycle has been shown to occur in a broad number of physiological conditions (22). Because the control of circulating fatty acids is a critical factor in the regulation of glucose oxidation by muscle, the triglyceride/fatty acid cycle has been implicated in Type 2 diabetes. It also turns out that the liver is a critical factor in triglyceride/fatty acid cycling and has a very high rate of glyceroneogenesis (23). Satish Kalhan (24) demonstrated that the liver of over-night fasted humans uses glyceroneogenesis almost exclusively to make the glyceride-glycerol for triglyceride synthesis. To support the general importance of glyceroneogenesis in adipose tissues, Lea Reshef and her student Yael Olswang (25) reported that the tissue-specific ablation of PEPCK-C gene transcription in adipose tissue of mice caused lipodystrophy, and Franckhauser et al. (26) found that the overexpression of PEPCK-C in adipose tissue resulted in obesity. Thus, glyceroneogenesis is critical for the control of fatty acid re-esterification during fasting and PEPCK is central to this pathway. In April 2003, a symposium devoted to glyceroneogenesis was held in Paris, France. This event was organized by Claude Forest and Elmus Beale, both of whom have made important contributions to the establishment of glyceroneogenesis in adipose tissue (31, 32). With this renewed interest, glyceroneogenesis is gradually being included in textbooks of biochemistry (27, 28).

Early Forays into Molecular Biology

Our finding of the rapid appearance of PEPCK-C activity in the liver at birth led us to determine whether this was due to the initiation of gene transcription or to regulation of the turnover of either PEPCK-C mRNA or the enzyme itself. By the early 1970s, techniques were becoming available to determine the levels of mRNA for specific proteins. Although the techniques were not available to isolate the gene for PEPCK-C (this came a decade later), it was possible to measure the synthesis rate of the enzyme using an antibody. John Ballard and I purified PEPCK-C from rat liver and developed a polyvalent antibody that we use to this day (29). With the antibody in hand, we carried out pulse-chase experiments to determine the effect of diet and hormones on the synthesis rate of hepatic PEPCK-C and to determine its half-life (about 6 h) (30). We also assessed alterations in the synthesis rate of PEPCK-C in the liver during development. At birth, the synthesis of PEPCK-C increased from negligible levels in rats in utero to adult levels within 12 h after birth; we could detect no degradation of the enzyme during this period of rapid enzyme synthesis. A parallel set of experiments with adult animals demonstrated that hormones such as glucagon and glucocorticoids increased the synthesis of PEPCK-C in the liver and that insulin decreased the rate of enzyme synthesis. A major player in this early research on the control of PEPCK-C gene expression was Shirley Tilghman, who was a graduate student in my laboratory (31). Shirley is now the President of Princeton University, no doubt the consequence of working on PEPCK early in her career!

From these early studies, it was apparent that the rate of synthesis of PEPCK-C was changed rapidly by diet and hormones and that these changes were very large in magnitude (i.e. 10-fold in 30 min). Studying the control of the expression of the gene for this enzyme seems in retrospect to be a “no brainer.” However, at the time there were other enzymes in which gene expression was altered equally rapidly by diet and hormones. These include malic enzyme, tyrosine aminotransferase, and glucose-6-phosphatase, to name just a few. Alan Goodridge (32, 33) had demonstrated that in chickens the initial expression of the gene for hepatic malic enzyme was induced at hatching by feeding carbohydrate. Both tyrosine ami-
notransferase and glucose-6-phosphatase were induced dramatically at birth in the livers of rats, and the expression of the genes for both enzymes was altered in the livers of adult mammals by hormones such as dexamethasone, glucagon, and insulin. We now know that there is a single copy of PEPCK-C in the mammalian genome, that it is a relatively small gene (7 kb), that its mRNA is relatively abundant, and that changes in gene transcription induced by diet and hormones are rapid and very large. The genes for the enzymes mentioned above turned out to be either difficult to isolate (glucose-6-phosphatase) or to have a simpler regulation of their gene transcription (tyrosine aminotransferase and malic enzyme). Our decision to invest the time and effort into the study of the control of transcription of the gene for PEPCK-C was critical in developing what is now the model for studying glucose-regulated gene transcription. Why did we choose to study PEPCK-C gene expression? There are two answers to this question: 1) we considered this enzyme to be of exceptional metabolic importance and thus worth the effort or 2) we did not know any better. The latter is no doubt closer to the truth since, as Shakespeare once said, “Fortune brings in some boats that are not steered.”

With some trepidation in the mid-1970s we began to isolate and determine the level of what was called at the time “translatable RNA” for PEPCK-C from the livers and kidneys of rats. I had no training in the isolation of RNA and very little understanding of molecular biology in general. My graduate education had been largely compartmentalized; I knew a lot about metabolism but had not a clue about the function of genes. Because there was no cDNA for a gene of interest available at that time, the levels of a specific mRNA involved the isolation of poly(A)+ mRNA from a target tissue and its subsequent translation in vitro by a wheat germ extract, using a radioactive amino acid. An antibody was then used to isolate the protein being studied, which was separated by gel electrophoresis and quantified. The amount of label in the isolated protein was equivalent to the amount of mRNA for the protein that has been added to the translation system. This was a laborious process that involved the preparation of wheat gene extract from batches of wheat germ that seemed to have magical properties that were difficult to reproduce from batch to batch. Today, no one ever refers to “translatable RNA” as a method to quantify the level of mRNA in a tissue, but for a time in the early 1970s, preparing a viable wheat germ extract was a skill of importance!

As luck would have it, Patrick Iynedjian, a Swiss physician-scientist, was doing a postdoctoral fellowship in my laboratory at the Fels Research Institute. Patrick's research interest was understanding the regulation of metabolic acidosis and its connection to renal gluconeogenesis, a process that involved the induction of PEPCK-C gene expression (34, 35). He showed clearly for the first time that metabolic acidosis and glucocorticoids increased both the rate of PEPCK-C synthesis and the level of PEPCK-C mRNA in the kidney cortex of rats (36, 37). At about the same time, Dimitris Kioussis (38) and Predez Garcia-Ruiz (39) studied the effect of cAMP on the induction of PEPCK-C mRNA in the liver. They also studied the effect of insulin on changes in hepatic PEPCK-C mRNA during development. What was surprising to me at the time was the rapidity of the changes in PEPCK-C mRNA (10-fold increase in hepatic mRNA in 30 min) and the fact that they paralleled the alterations that we had noted in the synthesis rate of PEPCK-C. Clearly, gene transcription and the rate of PEPCK-C synthesis from its mRNA were coordinated. At about that time, papers began to appear reporting the isolation of cDNAs for specific gene products. It had, at last, become possible to study gene transcription without indirectly measuring the concentration of an mRNA in a functional assay.

**Isolation of the Gene for PEPCK-C and the Characterization of the Metabolic Control of Its Transcription: an Ongoing Endeavor**

In 1978 I left Temple University to become the Chairman of the Department of Biochemistry at Case Western Reserve University School of Medicine in Cleveland, a position that I held until 1999. As I moved my laboratory to Cleveland, I was acutely aware of the need to clone the cDNA PEPCK-C and to use it to isolate and characterize its gene; without this there would be no progress in determining the mechanism of hormonal control of PEPCK-C gene transcription (and no grants). In retrospect, this was the most critical point in my foray into molecular biology. If we were unable to isolate the cDNA for PEPCK-C over a single granting cycle there would be no chance for renewal; needless to say, the pressure was on us. The approach used to accomplish this in the mid-1970s was to isolate the protein of interest, determine a portion of its amino acid sequence, and from that predict mRNA sequence coding for the protein. With
this in hand, it was possible to synthesize a series of probes to be hybridized against a cDNA library prepared by reverse transcribing hepatic mRNA. There were so many places that this procedure could go wrong that it required all of the optimism I could muster to keep from sinking into a state of deep depression when experiment after experiment turned out to be negative. It is hard to imagine the difficulty of this research today when almost any cDNA can be purchased!

We used the PEPCK-C that we had isolated from rat liver and were able to get a partial sequence that we used for the design of our probes. After many tries and a lot of heartache by several postdoctoral fellows, Heeja Yoo-Warren, a graduate student in my laboratory, isolated a putative cDNA for PEPCK-C from a library made from poly(A)$^+$ mRNA from the livers of diabetic rats. This work was published in 1981 (40). The gene was quickly isolated and the number of exons and introns in the PEPCK-C gene was determined by R-loop mapping. We found the number to be 8 (41); there are actually 9 (42). It turned out that this technique was not sensitive enough to detect a small intron at the 3'-end of the gene. At about the same time, Darryl Granner and his colleagues, then at the University of Iowa, published the sequence of the gene for PEPCK-C from the rat (42). Anyone who lived through this period can testify to the frustration in isolating a cDNA for a protein like PEPCK-C. There was no middle ground; you had a cDNA or you did not! It was very much like being in the neck of a bottle; inside the neck your research was totally constrained; once through the neck the possibilities seemed endless.

I have always had an interest in the metabolic role of PEPCK in different vertebrate animals (see Ref. 43 for more details of this interest). To this day, the metabolic roles of the two isoforms of PEPCK are poorly understood. To provide some information on this subject, we set about to characterize the genes for both isoforms of PEPCK-C from the chicken. John Cook isolated and sequenced the cDNA PEPCK-C (44), Sharon Weldon the cDNA PEPCK-M (45) from the chicken, and Yaccov Hod (46) characterized the gene for PEPCK-C from the chicken. The two isoforms of PEPCK have similar molecular masses (about 68 kDa), but they have only 60% sequence identity (45). In addition, transcription of the gene for PEPCK-M is not inducible by factors that stimulate the expression of the gene for PEPCK-C, such as cAMP (45). Clearly more work is needed to improve the characterization of both the biological role of the two isoforms of PEPCK, as well as the factors that control the transcription of PEPCK-M.

Once the gene for PEPCK-C was isolated, the action shifted to the characterization of its regulation by more direct techniques. Wouter Lamers and Herman Meisner (47) determined the effect of diet and hormones on the rate of gene transcription in the liver of rats using “run off” transcription analysis. We were very pleased to see that the rate of change of PEPCK-C gene transcription was rapid and the change was large. The half-time of transcription was 30 min after feeding carbohydrate to the rats, and the injection of Bt$_2$cAMP caused a 10-fold induction of gene transcription in 30 min. Thus, changes in the rate of transcription of the gene for PEPCK-C parallel the changes in the level of its mRNA and in the rate of enzyme synthesis. The rapidity of changes in PEPCK-C gene transcription in response to hormones has been a major asset in studying the control of this process in mammals. In fact, transcription of the gene is more rapid than RNA processing. Maria Hatzoglou (48), then a graduate student in my laboratory, found that the nuclei from rat liver, stimulated by the administration of Bt$_2$cAMP, contained a number of precursor species of PEPCK-C mRNA in various stages of processing; introns could be detected in the nucleus that had not yet been degraded. We even proposed a model for the maturation of PEPCK-C mRNA that involved the splicing of individual exons of the RNA that proceeds from the 5'- to 3'-end of the message.

Transcriptional analysis is often referred to, with some derision, as “promoter bashing.” My colleagues and I have spent almost two decades trying to understand the regulation of PEPCK-C gene transcription, so I know well the effort required to follow the every increasing complexity of gene transcription. Our work in this area, much of it in collaboration with Lea Reshef, began when the promoter became available for analysis in 1983. At the time, we had no idea of what to expect of a regulatory domain and where it might reside in the promoter, and no models were available to guide us. In a series of studies in the mid-1980s, we demonstrated that a relatively short segment of the PEPCK-C gene promoter contained all of the information needed to account for the induction of gene transcription from the promoter by cAMP and glucocorticoids and identified the first cAMP regulatory element ever described. I recall at the time being surprised that it was so close to the start site of gene transcription (-80).
Heidi Short (49), Tony Wynshaw-Boris (50, 51), and Jin-Song Liu (52) did a great deal of the early research that described the functional organization of the PEPCK-C gene promoter. Of special importance was the demonstration by Bill Roesler of the protein-binding domains in the promoter (53). Bill used DNase I footprinting to analyze ~1 kb of the gene promoter and identified a number of binding sites that are being tested for functional significance to this day. The number and proximity of binding sites and the apparent high affinity of transcription factor binding has made the PEPCK-C gene promoter particularly interesting for transcriptional studies (see Ref. 54 for a review). Dwight Klemm (55) carried out an elegant study that demonstrated the possibilities of using the PEPCK-C gene promoter for studies of in vitro transcription, an area still in need of careful study.

This research was quickly followed by a series of studies that identified a number of transcription factors that are involved in the hormonal and dietary control of PEPCK-C gene transcription. Of special importance was the demonstration by Bill Roesler of the protein-binding domains in the promoter (53). Bill used DNase I footprinting to analyze ~1 kb of the gene promoter and identified a number of binding sites that are being tested for functional significance to this day. The number and proximity of binding sites and the apparent high affinity of transcription factor binding has made the PEPCK-C gene promoter particularly interesting for transcriptional studies (see Ref. 54 for a review). Dwight Klemm (55) carried out an elegant study that demonstrated the possibilities of using the PEPCK-C gene promoter for studies of in vitro transcription, an area still in need of careful study.

This research was quickly followed by a series of studies that identified a number of transcription factors that are involved in the hormonal and dietary control of PEPCK-C gene transcription. Of special importance is the role of C/EBPα in the control of PEPCK-C gene transcription, first described in my laboratory by Bill Roesler and George Vandenbark (53) and Jin-Song Liu (52) and the role of C/EBPβ discovered by Edwards Park (56). Austin Gurney (57) demonstrated that c-Fos inhibited PEPCK-C gene transcription as effectively as insulin, Marta Giralt (58) mapped the thyroid hormone regulatory element in the PEPCK-C gene promoter, and Deborah Crawford and Patrick Leahy (59, 60) studied the inhibitory role of nuclear factor 1 on PEPCK-C gene transcription. More recently, Kaushik Chakravarty (61, 62) has studied the role of SREBP-1c in the insulin control of PEPCK-C gene transcription.

Fig. 3 summarizes the current version of the PEPCK-C gene promoter with the various transcription factors and co-activators that have been shown to bind to the promoter and/or contribute to the control of gene expression. These transcription factors interact with co-activator and co-repressors (SRC-1, PGC-1α, and CBP/p300) to form transcription complexes that control polymerase II activity. The various hormones and/or control factors (i.e. acidosis, alkalosis, fatty acids, glucose) initiate signaling cascades that recruit or activate transcription factors, which bind to specific sites on the PEPCK-C gene promoter. The complexity of the tissue-specific and hormonal regulation of PEPCK-C gene transcription is evident from Fig. 3; clearly, more work needs to be done before the control of PEPCK-C gene transcription is fully understood. However, the interested reader is directed to a recently published review of the mechanistic basis for the complex regulation of transcription of this interesting gene (63).

Most of molecular biology is carried out using transformed cell lines to study gene expression. This approach is a powerful one but has its limitations when one attempts to understand
the physiological control of a gene involved in metabolism. A great example of this is the control of PEPCK-C gene transcription in liver and adipose tissue by glucocorticoids; transcription of the gene is stimulated by glucocorticoids in the liver but inhibited in adipose tissue. Mary McGrane (64, 65), Yash Patel (66), Jed Friedman (67), and Pam Lechner (68) each carried out important studies of PEPCK-C gene promoter function by linking the promoter to a reporter gene and introducing this gene into transgenic mice. By introducing specific mutations into the gene promoter they tested the function of individual elements and determined the regions required for the tissue-specific transcription of the PEPCK-C gene. We also used this technique to test, in the intact animal, the putative hormone response elements that had been identified by promoter analysis using hepatoma cells in culture. My motto has always been that the intact animal is the metabolic “court of last resort”; if the promoter does not work as predicted when introduced into animals, there is something wrong with the prediction!

Lea Reshef and her student Yael Olswang abolished the expression of the gene for PEPCK-C in adipose tissue by mutating the PPARγ2 binding domain in the gene promoter in the germ line of the mice (25). The resultant tissue-specific ablation of PEPCK-C gene expression only in the adipose tissue stresses the required utilization of this regulatory site in this tissue only. Thirty percent of these mice became lipodystrophic because of the absence of glyceroneogenesis in their adipose tissue, accentuating the central regulatory role of PEPCK-C in this pathway. Colleen Croniger (69, 70) and Parvin Hakimi studied the metabolic consequences of a deletion of the genes for C/EBPα and C/EBPβ and PEPCK-C in mice. At the current time my colleague, Jianqi Yang, has produced a mouse model in which the gene for C/EBPα can be ablated in adult mice within 3 days by the induction of transcription of the gene for Cre recombinase. At 12 days after the ablation of the gene for C/EBPα the mice lose interest in food and have a continuing decrease in weight. They die almost exactly 2 weeks later. Our goal in all of these studies has been to make animal models that provide us with insight into metabolic regulation.

What Goes Around, Comes Around; the Return of Metabolism

A strange thing has happened recently; trendy journals like Cell and Nature have discovered metabolism. I am pleased to see it, even if it is several decades overdue! With the alarming increase in obesity and diabetes throughout the world (obesity has displaced smoking as the number one health hazard in the United States) and with a diet-conscious public, metabolism is now difficult to avoid. However, the new metabolism is quite a different discipline from the traditional subject that I learned as a graduate student. It is now deeply routed in molecular biology and genetics and has the power of genome-wide analysis to identify candidate genes involved in multigene diseases, such as diabetes. I have been very impressed with the powerful tools for the study of metabolism that have been provided by molecular genetics; the return of metabolism to the “front burner” of the biomedical sciences has very much to do with the new techniques available to study old problems. Metabolism has always been an important part of medical education, and teaching metabolic principles to our medical students and undergraduate biochemistry majors has been an important part of my scientific life.

My Career as an Editor of the Journal of Biological Chemistry

Emerson wrote that: “To genius must always go two gifts, the thought and the publication.” My former boss, Emmanuel Farber, then the Director of the Fels Research Institute, said the same thing but more directly; he had a sign over his desk that read, “If it’s not published, it’s not research.” How true that statement is! My first publication was in 1960 in the Journal of Biological Chemistry (71), and since that time, I have never wanted to publish my work anywhere else. There is something about the Journal that has inspired me (and many others as well) to devote so much of our efforts (in my case 30 years) to its service. Almost every working day for the last 20 years I have spent several hours involved in some aspect of its business. Through all of this, I have always felt that I was involved with something far bigger than myself and that I was part of a great line of biochemists, many long past, who have worked to make the Journal a true icon of biochemistry. To me the Journal is the greatest institution in all of biochemistry; there is nothing even close to it for its critical contributions to our science. As an Associate Editor for the past 20 years, I have watched with great pride as the Journal has led the way in the world of electronic publishing and more recently in the totally electronic submission and review of manuscripts. For the past 40 years the Journal has been led by Herb Tabor; he is in the long tradition of dedicated and truly far-sighted individ-
Reflections: Metabolism in the Era of Molecular Biology

I have had the great good fortune to have worked for my entire scientific life studying the regulation of metabolism. Much of my work over my entire career has focused on the metabolic role of PEPCK. Early in my career, Mulchand Patel and I were interested in the metabolism of adipose tissue (72); today I still study the role of PEPCK-C in that tissue (22). With Koshinaka Ogata, Alan Garber, and Mireille Jomain-Baum, I studied the control of hepatic gluconeogenesis in various species (73, 74), and Ifeanyi Arinze and I worked together to determine the pattern of development of the two isoforms of PEPCK in the liver at birth (75, 76). The development of PEPCK-C is still an interest to me, but now I study the role of specific transcription factors in this process. In the 1990s my research turned to the area of gene therapy; my colleague Jose Perales developed a method for introducing genes into mammalian tissues (77). The fact that I am still active in research at the age of 69 is a testimony to the fact that steady, careful work on an important topic can lead to significant contributions; it is not necessary to be flashy, only steady. However, I have also found that nothing worthwhile in research can be accomplished alone. I have been blessed over the years with outstanding colleagues; there are too many to acknowledge in this article, but I thank them all for what they have given me. I especially have a deep debt of gratitude to John Ballard and Lea Reshef; they gave me much more than I ever gave to them. Over the past decade, my collaboration with Satish Kalhan has also shown me the importance of metabolic research with humans and how powerful it can be in studying the true nature of disease. All of my collaborators and students know all too well (as I do) that research is difficult and often discouraging; most experiments fail! I have always ascribed to Winston Churchill’s admonition that: “Success is going from failure to failure with enthusiasm.”

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REFERENCES

1. Ballard, F. J., and Hanson, R. W. (1967) J. Lipid Res. 8, 73–79
2. Soling, H.-D., and Wills, B. (eds) (1971) Regulation of Gluconeogenesis, Georg Thieme Verlag, Stuttgart, Germany
3. Hanson, R. W., and Fenton, P. F. (1965) Proc. Soc. Exp. Biol. Med. 121, 343–346
4. Weinhouse, S., Medes, G., and Floyd, N. F. (1944) J. Biol. Chem. 153, 143–151
5. Dipietro, D. L., and Weinhouse, S. (1960) J. Biol. Chem. 235, 2542–2545
6. Ballard, F. J. (1975) Methods Enzymol. 42, 43–47
7. Ballard, F. J. (1966) Biochem. J. 101, 70–75
8. Ballard, F. J. (1966) Biochem. J. 98, 347–352
9. Hanson, R. W. (1965) Arch. Biochem. Biophys. 109, 98–103
10. Utter, M. F., and Kech, D. B. (1960) J. Biol. Chem. 235, 17–18
11. Utter, M. F., and Kurahashi, K. (1953) J. Am. Chem. Soc. 75, 785–787
12. Nordlie, R. C., and Lardy, H. A. (1963) J. Biol. Chem. 238, 2259–2263
13. Ballard, F. J., and Hanson, R. W. (1967) Biochem. J. 104, 866–871
14. Ballard, F. J., Hanson, R. W., and Leveille, G. A. (1967) J. Biol. Chem. 242, 2746–2750
15. Gorin, E., Tad-Or, Z., and Shaffir, E. (1969) Eur. J. Biochem. 8, 370–375
16. Reshef, L., Niv, J., and Shapirio, B. (1967) J. Lipid Res. 8, 688–691
17. Reshef, L., Niv, J., and Shapirio, B. (1967) J. Lipid Res. 8, 682–687
18. Reshef, L., Hanson, R. W., and Ballard, F. J. (1970) J. Biol. Chem. 245, 5979–5984
19. Vaughan, M. J. (1962) J. Biol. Chem. 237, 3354–3358
20. Reshef, L., Meyuhas, O., Boshwitz, C., Hanson, R. W., and Ballard, F. J. (1972) Isr. J. Med. Sci. 8, 372–381
21. Jensen, M. D., Ekberg, K., and Landau, B. R. (2001) Am. J. Physiol. 281, E789–E793
22. Reshef, L., Olswang, Y., Cassuto, H., Blum, B., Croniger, C. M., Kalhan, S. C., Tilghman, S. M., and Hanson, R. W. (2003) J. Biol. Chem. 278, 30413–30416
23. Botton, L. M., Brito, M. N., Brito, N. A., Brito, S. R., Kettelhut, I. C., and Migliorini, R. H. (1998) Metabolism 47, 1217–1221
