BUILDING INTERLEVEL THEORIES: THE DISCOVERY OF THE EMBDEN-MEYERHOF PATHWAY AND THE PHOSPHATE CYCLE

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The 1930s were years of rapid advance in the biochemical study of intermediary metabolism. Investigators discovered the role of phosphates in energy transport, the function of coenzymes in group transport, and the tricarboxylic acid cycle. One thing that is common to all of these discoveries is that they involve recognition of cyclic processes in which, through a series of chemical reactions, a substance that appeared earlier in the pathway is reconstituted (see Krebs, 1947). While the idea of cyclic processes had arisen earlier (Meyerhof's lactic acid cycle will be discussed below; see Holmes, 1986, for an analysis of Thunberg's postulation of an acetic acid cycle), it assumed a new degree of prominence in the 1930s when it became recognized as a common mode of organization in the cell. I contend that the recognition of organization played a transforming role, revealing the existence of a level of organization in the cell between that of individual molecular reactions (involving enzymes and substrates) and that of physiological processes (providing the energy for muscle action). Had there not been this systematic structure in the cell, then the chemical level of explanation would ultimately have

sufficed - in an important sense, physiology would have been reduced to chemistry. The organization that was discovered in the 1930s, however, orchestrates the chemical events, and thus is itself an important ingredient that must be included in any explanation.

In this paper I will focus on the discovery of the role of phosphates in energy transport and inquire as to how the complex organized system of energy transport was discovered. The discovery of this aspect of biochemical organization in the cell contrasts with the discovery of another aspect of the organization, that involving coenzymes as agents of group transport. (I have explored this elsewhere; see Bechtel, 1984a and 1984b). In that case, the critical contribution was the discovery of the contributing chemical mechanisms (in particular, the discovery of reversibly oxidizable substances like NAD). Once Warburg discovered the nature of the chemical reaction of coenzymes, it was readily apparent what their function was. The discovery of the role of phosphates in energy transfer, however, is significantly different. Although the ultimate biochemical account incorporated numerous chemical mechanisms that were discovered prior to 1930, the function of phosphate did not simply emerge from these discoveries. Rather, major contributions were made by investigators whose attention was directed not just at chemical events but at the physiological roles these events were to serve. As a result of this dual focus, these investigators often advanced models of how the chemical activities might accomplish physiological functions. These models were sometimes premature and speculative; some were simply wrong. However, from this model building effort some of the major insights into the energy transferring function of phosphates and their role in providing systemic functional organization emerged.

The case is of philosophical interest in part simply as a case of scientific discovery. The task of understandin the process of scientific discovery has assumed new interes in philosophy of science in recent years as philosophers have recognized that in actual scientific endeavors the contexts of discovery and justification cannot be distinguished so neatly as thought by a previous generation Although one cannot aspire to a logic of disvovery in the sense of a procedure that can simply generate new scientifi truths (this was a false goal in any case, since neither can one aspire to a logic of justification capable of justifying all and only true scientific propositions), one can hope to distinguish among the strategies scientists have employed those that have been successful and those that have led to errors (I have pursued this approach in Bechtel, 1982). But the interest in this case goes much further, for it involves discoveries concerning the relation between different levels of organization in nature. For philosophers, theory reduction has provided the basic model for such connections, but as numerous case studies have shown (Darden and Maull, 1977; Maull, 1977, Bechtel 1984a and 1986b), actual scientific research programmes have employed a quite different conception of relations between levels of organization. This case offers additional perspective on the question of how scientists develop interlevel connections in their research.

My primary focus in this paper will be on the contributions of two investigators, Gustav Embden and Otto Meyerhof, each of whom offered a theoretical model in the early 1930s that proved critical in developing the understanding of the function of phosphates in energy transfer. In both cases, the framework was partly grounded in earlier empirical research but, as I shall try to show in discussing that research, it did not itself lead to the new models. Rather, Embden and Meyerhof extended their

focus beyond the chemical information that was available and focused on the character of the physiological problems they were confronting. Even though their work was often intertwined, their major contributions can be analyzed separately. Part 1 will therefore focus on Embden's development of a model of the glycolytic pathway that included phosphorylated intermediaries while part 2 will analyze Meyerhof's development of a scheme of chemical coupling through phosphate transfer. In part 3 I will briefly explore how these two schemes were put together and the chemical account filled in so that by the end of the decade a coherent understanding of the phosphate cycle had developed. Finally, I will try to put this case in philosophical perspective in part 4, exploring how the philosophical model of theory reduction is inadequate to the case and how one can better understand the research endeavors in terms of Darden and Maull's notion of an interfield theory. I will also consider the somewhat different conception of reduction used by scientists, which figures in what Richardson and I (forthcoming) call localizationist research programs. Identifying functional units that contribute to producing the overall function is an important part of scientific explanation, but it is not the only part. I will conclude by considering how much localizationist research contributed to the final understanding of the role of phosphates in energy transfer, and what else was involved in developing this account.

Prior to turning to the contributions of Embden and Meyerhof it will be useful to give additional historical background that will provide some perspective on the later history. In the 19th century one finds two distinct approaches to explaining metabolic phenomena like fermentation. The chemical approach took fermentation to be a purely chemical phenomenon and sought to identify chemical agents responsible for it. The inspirations for

this programme, which flourished primarily around the middle of the century, were the success in identifying chemical agents responsible for other cell functions (e.g, Payen and Persoz's discovery of diastase as an agent for converting starch into sugar) and Berzelius' articulation of the concept of a catalyst as a chemical agent instrumental in facilitating reactions in other materials without being changed itself. Liebig, Traube, and Hoppe-Seyler and Kühne, despite their differences, were all champions of this approach. (Kühne introduced the term "enzyme" for intracellular molecules responsible for catalyzing cellular functions.) In the latter part of this century this approach was overshadowed by the biological tradition. Some in this tradition adhered to Schwann's and Pasteur's arguments that only living cells were able to achieve fermentation. Others like Pflüger and Haeckel took the protoplasm that comprised the cells to be a special chemical substance and tried to explain physiological reactions as resulting from the special constitution of this substance. The common characteristic of the biological tradition was an emphasis on special laws that governed phenomena in living systems. (See Teich, 1981, for an overview of the 19th century fermentation controversies. I have discussed the various late 19th century approaches to metabolism in Bechtel, 1984b).

The domination of the biological tradition was broken in 1897 when Buchner accidentally succeeded in producing fermentation in material extracted from cells. By attributing fermentation to an intracellular enzyme which he called "zymase," Buchner gave new life to the chemical tradition. (See Kohler, 1971 and 1973, for discussion of the character and significance of Buchner's research.) While Buchner's demonstration with yeast juice was a major factor in the overthrow of the biological tradition, his interpretation of the result in terms of a single enzyme zymase was a

view oversimplified in the opposite direction. Neumeister ans Wroblewski, amongst others, argued that the cell contained numerous enzymes that contributed to fermentation. However, since they linked their views with the protoplasm tradition, proposing that these enzymes were linked into complex structures, their views were repudiated by Buchner and others who now saw the way open to a purely chemical account of fermentation. (See Florkin, 1975, Chapter 17 for details).

What I will be showing in this paper is how biochemists ultimately had to reject this purely chemical perspective and recognize a higher level of cellular organization as critical to the performance of physiological activities like fermentation. One way to view the discoveries of the 1930s is as showing that the conceptual orientation (as distinct from the actual theories) of 19th century researchers was appropriate - that physiological processes are not realized through chemical processes alone, but through highly organized sequences of chemical processes. In this sense, the history I will be discussing here can be viewed as repeating a pattern found in the 19th century as well.

1. EMBDEN'S INTRODUCTION OF PHOSPHORYLATED INTERMEDIARIES IN GLYCOLYSIS

Buchner's work on fermentation is directly relevant to the material to be discussed in this section. Although earlier some researchers had anticipated a connection between the processes of alcoholic fermentation and muscle glycolysis (lactic acid fermentation), they were generally treated separately before 1920. Gradually, however, researchers discovered that the chemical reactions of the two processes are nearly identical, typically by replicating

in muscle extracts what had already been done in yeast extracts. Since it provided the basis for later work on muscle glycolysis, in the early part of this section I will be discussing work done on alcoholic fermentation.

Above I noted how Buchner initially interpreted fermentation in terms of the activity of a single enzyme, zymase. However, it was soon established that a single enzyme could not account for the whole process. The rate of fermentation in yeast juice dropped dramatically after a few minutes. In trying to account for this, Harden and Young (1906) established that a co-enzyme that was heat-stable, dialyzable, and percipitable by alcohol was essential for the functioning of zymase. As important for the purposes of this paper is the fact that Harden and Young also followed up on the discovery of Wroblewski that inorganic phosphate had to be added to the fermentation solution to continue the reaction. They established that after a short time a fermenting solution settled to a slow steady rate and that adding inorganic phosphate resulted in a temporary increase in this rate followed by a return to the lower level. The additional carbon dioxide produced in the rapid phase was shown to be proportional to the increased phosphate.

Harden and Young also found that the added phosphate was taken up into a non-percipitable ester, hexosediphosphate, which only fermented very slowly. As a result, they concluded that hexosediphosphate could not figure centrally in the pathway of fermentation but must somehow be a biproduct. In 1908 they proposed a theory according to which the combination of a hexose molecule and two phosphates made another hexose molecule more labile, causing it to break down into two triose molecules and to continue along the pathway. Since it appeared that the ester itself could not be fermented, however, they proposed that it had

to be hydrolyzed and release its phosphates (a slow process) before the sequence could repeat itself. (See Figure 1.) The rapid fermentation when phosphates were added to the solution was due to there being an adequate supply of inorganic phosphate for the first of these reactions to proceed. The reaction slowed down, however, when this phosphate was used up and the reaction had to await the hydrolysis of a hexosediphosphate. (For further discussion of this research, see Kohler, 1974.)

Glycolytic reaction:

$$2\ C_6H_{12}O_6 + 2\ R_2HPO_4 \rightarrow 1\ C_6H_{10}O_4(PO_4R_2)_2 + 2\ C_2H_5OH + 2CO_2 + 2H_2O$$

Hexose phosphate hexosediphosphate alcohol

Hydrolysis Reaction:

$$1\ C_6H_{10}O_4(PO_4R_2)_2 + 1H_2O \rightarrow 2\ C_6H_{12}O_6 + 2\ R_2HPO_4$$

hexosediphosphate hexose phosphate

Figure 1. Harden and Young's Conception of the role of phosphates in glycolysis.

Harden and Young were mislead by the failure of hexosediphosphate to ferment rapidly in their artificial solutions, which is actually due to the destruction of crucial enzymes in creation of the yeast extract. They interpreted the failure of hexosediphosphate to ferment in accord with what investigators in this area took as a standard maxim: that nothing could be an intermediary in fermentation that could not be fermented as quickly as sugar itself. This maxim seems entirely reasonable but, as in this case, could lead researchers astray when they were dealing with systems involving more than simply a linear sequence of catabolic reactions.

Having excluded phosphates from the pathway, fermentation researchers went on to try to identify the intermediate substances that were formed in the sequence of fermentation reactions. The attempt to identify intermediary substances was guided by earlier, purely chemical endeavors to transform sugar to alcohol through, for example, the action of alkalis. In that process three three-carbon compounds - glyceraldehyde, methylglyoxal, and lactic acid - were taken to be intermediates. Much of the experimentation in the first decade of this century investigated whether these substances figured in the fermentation process as carried on in living organisms. One of the principal tests was whether these substances could be fermented; glyceraldehyde as well as two other substances, dihydroxyacetone and pyruvic acid, were shown to meet this test. It was also established that pyruvic acid was produced in the course of fermentation (by using a fixing agent to remove it from the preparation).

Theorists tried to use the results of these studies as well as knowledge of possible chemical reactions to devise accounts of the complete pathways of reactions involved in going from glucose to alcohol. Numerous schemas were proposed during the first two decades of the century (see Harden, 1932, for a review of many of them), but the one that gained the most acceptance was offered by Neuberg and Kerb (1914). It proposed a scissioning of the glucose molecule into two molecules of methylglyoxal. Initially, they proposed, these two undergo a Cannizaro reaction, oxidizing one molecule to pyruvic acid and reducing the other to glycerol. The pyruvic acid is then decarboxylated, yielding acetaldehyde. Subsequently, it is the acetaldehyde that participates in the Cannizaro reaction, being reduced to alcohol while the methylglyoxal is oxidized to pyruvic acid. (See Figure 2. Note that in this scheme a kind of cyclic process occurs. The motivation for proposing this

cycle was purely chemical - its oxidation of methylglyoxal had to be balanced by a reduction, and one already required a reduction of pyruvic acid to lactic acid.)

(1) $C_6H_{12}O_6 \xrightarrow{2H_2O} 2\ C_3H_4O_2$
Hexose — Methylglyoxal

(2a) $2\ C_3H_4O_2 + 2H_2O \rightarrow C_3H_8O_3 + C_3H_4O_3$
Methylglyoxal — glycerol — pyruvic acid

(2b) $C_3H_4O_2 + C_2H_4O + H_2O \rightarrow C_3H_4O_3 + C_2H_5OH$
Methylglyoxal Aldehyde — Pyruvic Acid Alcohol

(3) $C_3H_4O_3 \rightarrow C_2H_4O + CO_2$
Pyruvic Acid — Aldehyde

Figure 2. Glycolytic Pathway Proposed by Neuberg and Kerb.

Besides providing a complete sequence of reactions, this model could account for the methyl that had been found amongst the products of fermentation. However, there was a strong argument against the inclusion of methylglyoxal - it violated the maxim noted above that all intermediaries had to be fermented as rapidly as glucose itself. Methylglyoxal could not be fermented in living cells. In this case, experimenters excused the failure as an artifact of trying to add a substance to the cell experimentally rather than having it produced in the cell. For twenty years the Neuberg and Kerb model was highly regarded and thought to provide the correct account of fermentation.

The schemes of Harden and Young and of Neuberg and Kerb both turned out to be wrong. The phosphates that

Harden and Young excluded from the pathway figure throughout the process and play a critical role in the process. It was Embden who introduced the idea of phosphorylated intermediaries. As noted above, this early work I have been discussing focused on alcoholic fermentation. Embden, in his early research tried to replicate with muscle extract the work of Buchner on cell-free fermentation. He established that lactic acid increased in the muscle extract over time, but adding glucose failed to increase the yield (Embden, Kalberlah, and Engel, 1912). Embden hypothesized that the lactic acid that was formed in the preparation was not derived directly from glycogen but from an unknown precursor which he called "lactacidogen." It was at this stage that Embden called on a crucial physiological observation that muscle work resulted in an increase in phosphoric acid. He discovered that lactic acid and phosphoric acid were produced in his muscle juice in equimolar proportions, suggesting that lactacidogen was the precursor of both substances. He thus tested adding the hexosediphosphate ester of Harden and Young to his muscle juice and found that it increased the production of lactic acid, leading him to infer that it might be lactacidogen (Embden, Griesbach, and Schmitz, 1914).

There were two significant parts of Embden's contribution. One was to show that muscle glycolysis resembled fermentation in that both could be accomplished in cell-free extracts. Meyerhof further secured this parallel in 1925 when he extracted an enzyme system from muscle cells that succeeded in converting glycogen to lactic acid. Meyerhof earlier had found a further parallel between the two cases when, in 1918, he established that the coenzyme Harden and Young had shown to be necessary for fermentation was also generally present in animal cells.

There was, however, another side of Embden's contribution - the hypothesis of a phosphorylated precursor of lactic acid. Although there were numerous investigations into this proposed precursor, most of the researchers working on muscle as well as alcoholic fermentation accepted the Neuberg and Kerb model that incorporated methylglyoxal, a non-phosphorylated substance, as the major intermediary in the formation of pyruvix acid. As we shall see, though, Embden was led to take seriously the idea that phosphorylated substances constituted the intermediaries in glycolysis. (It is worth noting that the observation that guided Embden's inquiry is known today to have a quite different explanation: the phosphate liberated in muscle activity comes mostly from phosphocreatine and ATP, not the phosphorylated precursor of lactic acid. The phosphate in the precursor is transferred directly to ATP.)

The researcher who came closest to accepting Embden's views that a phosphorylated substance was an intermediary in muscle glycolysis was Meyerhof. Already in Meyerhof (1924) he offered evidence that it was the phosphorylated hexose that split into trioses without first undergoing hydrolysis. Subsequently, he gained further evidence by using fluoride as a poison to inhibit glycolysis and producing a build up of hexosediphosphate. But Meyerhof confronted the same obstacle as had others faced - hexosediphosphate could not be fermented in artificial solutions. Meyerhof (1930) proposed a way around this objection that treated a special form of hexosephosphate (an "active" form) as an intermediate. One molecule of the active form would pass its phosphate to the other, creating hexosediphosphate as "a stabilization stage," while the other split into two trioses. The hexosediphosphate itself would slowly split into two monophosphates that could reenter the pathway. Meyerhof admitted that this was only a hypothesis to account for the physiological data and that he lacked a chemical interpretation of the notion of a stabilization stage.

In the early 1930s Embden took a step far beyond Meyerhof or other researchers investigating the pathway of glycolysis, proposing a model which incorporated the conviction that phosphorylated substances were the precursors of lactic acid. He replaced the intermediaries proposed by Neuberg and Kerb by their phosphorylated forms. According to this scheme, hexosediphosphate is scissioned into two triosephosphates. One of these triosephosphates is oxidized to 3-phosphoglyceric acid at the expense of reducing the other to glycerophosphoric acid. The 3-phosphoglyceric acid is next dephosphorylated to pyruvic acid; pyruvic acid is then reduced to lactic acid, reoxidizing glycerophosphoric acid to triosephosphate (Embden, Deuticke, and Kraft, 1933). (See Figure 3.)

(1) $C_6H_{10}O_4(PO_4H_2)_2 \rightarrow 2\ C_3H_5O_2(PO_4H_2)$
Hexosediphosphate — triosephosphate

(2) $2\ C_3H_5O_2(PO_4H_2) + H_2O \rightarrow C_3H_5O_3(PO_4H_2) + C_3H_7O_2(PO_4H_2)$
triosephosphate — 3-phosphoglyceric acid — glycerophosphate

(3) $C_3H_5O_3(PO_4H_2) \rightarrow C_3H_4O_3 + H_3PO_4$
3-phosphoglyceric acid — pyruvic acid — phosphate

(4) $C_3H_4O_3 + C_3H_7O_2(PO_4H_2) \rightarrow C_3H_6O_3 + C_3H_5O_2(PO_4H_2)$
pyruvic acid — glycerophosphate — lactic acid — triosephosphate

Figure 3. Glycolytic Pathway Proposed by Embden et al.

Embden did have some empirical evidence to support this radical proposal. What is significant, is that it had already been discovered by other investigators who, however, had placed more traditional interpretations on it.

In 1930 Lipmann and Lohmann had produced from hexosediphosphate under fluoride poisoning an ester that was very difficult to hydrolyze. In the same year Nelsson identified 3-phosphoglyceric acid, which he, however, took only to be a side-product of alcoholic fermentation. Although they were in communication, Nelsson and Lohmann and Lipmann failed to see the connection between the esters they had examined. What Embden's scheme did was to interpret Lipmann and Lohmmann's ester as a combination of two phosphorylated 3-carbon compounds from which 3-phosphoglyceric acid was then produced by oxidation. It thus offered a new perspective on the overall process.

Embden did not appreciate the significance of having phosphorylated intermediaries throughout the glycolytic pathway. That contribution came from Meyerhof. Moreover, Embden lacked empirical support for many aspects of his scheme. That, however, does not lessen the significance of his accomplishment, which was to put the chemical events into a coherent framework. Lipmann comments on Embden's accomplishment: "Although in these Embden experiments the glycerophosphate was not found and their experimental background was quite sketchy, they managed to piece together, courageously and with great vision, the first coherent scheme of the oxidoreductive events that followed the scission of FDP [hexosediphosphate]" (Lipmann, 1971, p. 15).

This endeavor to piece together data into a coherent framework was a characteristic of Embden. A frequent critic of Embden's, Parnas, commented on Embden's endeavors to provide a coherent model of the phenomena he was studying in his obituary to Embden (who died very soon after proposing the model of glycolysis we have just been discussing): "Embden was, to use the phrase employed by Ostwald and Smoluchowski, a romantic explorer: very bold ideas, arising sometimes before, sometimes after his observations, gave

him a picture of the process, sometimes down to minute particulars, and this picture was then tested by ample experimental work, not always careful and critical enough, but always very fertile and leading to further experiments and consequences. Much of this work and many of his results have been swept away by the further development of research: others, however, have become outstanding facts and ideas of biochemistry" (Parnas, 1933). As Parnas notes, the models Embden constructed often proved incorrect, but the general tendency to develop such models to account for physiological phenomena was a critical ingredient in ultimately coming up with adequate theories. The data was available, what was needed was the critical idea to construe glycolysis as proceeding through phosphorylated intermediates rather than simpler substances like methylglyoxal. The physiological detail that phosphorus is released in muscle activity guided Embden's thinking in a quite different direction than those simply trying to work out chemical pathways.

2. MEYERHOF'S CONTRIBUTION IN CONNECTING CHEMICAL EVENTS WITH MUSCLE FUNCTION

Although a number of the investigations discussed in the previous section were performed on muscle cells or muscle juice, they did not draw explicit connections between these metabolic processes and the work of muscle. However, other investigations tried to establish such connections. In this section I will examine the line of research that led to recognizing the connection between phosphoric esters and muscle work. Up until the late 1920s, however, the main line of research did not suggest any such role for phosphorus compounds, as it tended to point to a direct connection between the formation of lactic acid in muscle cells and muscle work.

During the 19th century numerous investigators had identified lactic acid in muscles in the state of rigor mortis, but it was Fletcher and Hopkins (1907) who provided the definitive demonstration that lactic acid accumulates during anaerobic muscle contraction and that it disappears during oxidative recovery. Subsequent years saw detailed quantitative studies of the relation between glucose levels, lactic acid levels and the response of these to oxygen. These studies were coupled with investigations into the relation of the chemical events in the cell and the heat production, which were carried out principally by Hill and Meyerhof. Initial measurements indicated three important facts: that heat production is greater during oxidative recovery than during anaerobic contraction, that the heat produced during contraction is greater than that theoretically available in the production of lactic acid from glycogen, and that the heat produced during recovery is less than would be expected on the basis of complete oxidation of lactic acid.

Although a number of proposals were made during the period 1910-1920 to explain these facts, Meyerhof's proposal of a lactic acid cycle provided the first apparently adequate explanation of them. According to this proposal approximately three-fourths (the ratio was altered under various circumstances) of the lactic acid produced in anaerobic contraction is reconstituted into glycogen during recovery. Meyerhof postulated that the energy for resynthesis came from coupling that process with the oxidation of the smaller portion of lactic acid (see Meyerhof, 1924, for further discussion). Previously, all the lactic acid was thought to be oxidized during recovery. This cycle is important for a couple reasons: it represents one of the early cases where a more complex mode of organization was posited in place of a simple catabolic model in order to handle the physiological data and where a coupling of

reactions was postulated simply to handle the thermodynamics without knowledge of the mechanism for coupling. The lactic acid cycle, however, could not account for the additional heat produced during anaerobic contraction; Hill, Meyerhof, and others offered numerous proposals in succeeding years to try to find a source for this heat.

Information about thermodynamics of muscle provided important constraints on the theorizing of biochemists. Thus, Needham comments: " Such measurements of heat production, whilst telling us nothing about the actual chemical reactions going on, are most valuable to the biochemist, in indicating at what stage such reactions are to be expected. If some idea of the nature of these reactions is already entertained, then light can be thrown on their extent and possibly on the interrelationships of different suspected reactions" (1971, p. 52). In the early days, however, the thermodynamic data was often perplexing, since at the time the ideas about the mechanisms were inadequate to account for the data. Part of the problem is that the energy for muscle contraction does not come directly from glycolysis. A hint to this effect came from the work of Embden, who offered evidence as early as 1924 that not all the lactic acid formation occurred in the course of muscle contraction, but that a significant amount occurrred in the period after contraction was complete. Meyerhof disputed this claim for a number of years, but by 1931 came to accept it (Meyerhof, 1931a).

The reason for the delayed appearance of lactic acid was that glycolysis was not the immediate source of the energy employed in muscle work, contrary to the general assumption of the time. Recognizing that, however, depended on identifying the function of two other compounds discovered in the late 1920s. Eggleton and Eggleton (1927) isolated a rapidly hydrolysable substance from the cell

that on hydrolysis released large quantities of phosphorus. At first this substance was called "phosphagen," but is now known as "phosphocreatine." Working from different starting points, both Lohmann (1929) and Fiske and Subbarow (1929) isolated another compound which was readily hydolyzed into adenylic acid and pyrophosphate. This substance subsequently came to be known as "adenosine triphosphate" (ATP). A crucial fact that was noticed about both of these substances was that on hydrolysis they released large quantities of heat. When they discovered this feature of phosphocreatine, Meyerhof and Lohmann (192) proposed that it might be the source of the missing heat of contraction, but subsequently retracted this proposal when they could not identify a source of energy for the resynthesis of phosphocreatine during recovery.

The recognition that these compounds were indeed the proximate agents of energy for muscle contraction resulted from work of Lundsgaard that was actually directed toward a different issue - amino acid metabolism. In the course of this work he injected rabbits with iodoacetate and discovered that it was highly toxic. The pattern of this toxicity was what was important, though. With muscular exercise, the animal entered rigor mortis but without the formation of lactic acid. Lundsgaard showed in extracted frog muscles poisoned with iodoacetate that muscular contractions prior to the unset of figor occurred without the formation of lactic acid but with the breakdown of phosphocreatine. He thus established that phosphocreatine could provide the energy for muscular contraction and suggested that it was also the source in normal muscle. (Lundsgaard, 1932, recounts this research.)

The scene was now set for Meyerhof to bring ATP into the account and piece together a picture of the role of phosphate bonds in providing energy for muscle work.

Meyerhof, Lohmann, and Meyer (1931) recognized that ATP is a critical component in the fermentation coenzyme identified by Harden and Young and in the coenzyme of lactic acid fermentation identified by Meyerhof. Since the coenzyme had previously been shown to be needed for the early steps of glycolysis, this suggested that ATP figured there too. Meyerhof and Lohmann (1932) further established that the complete hydrolysis of ATP (to AMP) released sufficient energy to synthesize two molecules of phosphocreatine, while glycolysis was capable of bringing about the resynthesis of ATP. They pieced these clues together to propose an integrated system in which phosphates would provide the linkage through several steps in the energetic process:

> ... the present experiments lay the foundation of the thesis that the endothermic synthesis of phosphocreatine can take place through a coupling of this process with the exothermic and spontaneous breakdown of ATP, whilst the resynthesis of ATP out of adenylic acid and inorganic phosphate is made possible through the energy of lactic acid formation. One may also assume here a coupling of the synthesis with the metabolism of the intermediate hexose esters and so see in the phosphate groups contained in all these compounds, the unique carriers of the chemical coupling process. This would at once make understandable how the ATP acts as co-enzyme of the lactic acid formation, in that the taking up of phosphate by the hexose stands in connection with the giving up of the phosphate on the part of ATP; or the giving up of the phosphate on the side of the ester is concerned with the rebuilding of the pyrophosphate on the adenylic acid. This conception would also make possible a special meaning for the fact that for the anaerobic splitting of hexoses intermediate esterification with phosphorylic acid is above all necessary." (Meyerhof and Lohmann, 1932).

This paper was published just prior to Embden's proposal of a glycolytic pathway in which the hexose-phosphate ester scissioned into two triose phosphates. Embden's proposed pathway permitted a simplification in Meyerhof's scheme, for the hexosephosphate did not have to relinquish its phosphate back to ATP before scissioning into trioses. In the next section I will briefly discuss the research that filled in the links between Embden's pathway and Meyerhof's scheme in which phosphates provided the means for energy coupling. Before doing that, however, one should notice the nature of the contribution Meyerhof had made at this point. Meyerhof's thinking here is following the same direction as when he proposed the lactic acid cycle. He is trying to put the chemical events into a coherent framework so as to explain physiological data. Meyerhof had been criticized (for example, by Hahn, 1931), for employing notions like energetic coupling or coupled reactions without providing a stoichiometric equation. Such a requirement had been required by Ostwald (1900) when he introduced the notion of coupled reactions that directly transfer energy as distinct from reactions which proceed independently, taking or imparting thermodynamic energy into the milieu in which the reaction occurs. Meyerhof (1931b) defended his use of the notion of coupling as explicitly vague, intended to characterize the nature of the overall process where there were, as yet, insufficient grounds to identify the determinate reactions. The 1932 paper with Lohmann offered a more specific characterization of the mechanism of linking, but still did not offer stoichiometric equations for the reactions. Subsequent research produced such equations, but what is worth noting is that Meyerhof's endeavor was to try to synthesize chemical and physiological knowledge into a coherent scheme prior to experiments that filled in the details. Such modeling was important, for it served to direct subsequent research by indicating the kinds of chemical processes that

one could expect if the overall scheme was correct. (For additional discussion of Meyerhof's research strategies by investigators who had worked with him, see the obituary by Nachmansohn, Ochoa, and Lipmann, 1952).

3. COMPLETING THE EMBDEN-MEYERHOF PATHWAY

In the previous two sections my focus has been on the model building endeavors of Embden and Meyerhof, one of whom developed a model of the glycolytic process that included phosphates as intermediaries and the other of whom proposed that phosphate bonds provided the connection between the various energetic processes involved in muscle work. These investigators built models that went beyond what had been experimentally determined but which put the experimental results into a coherent framework. These models also suggested further investigations. In this section I will briefly explain how research in the subsequent half-decade succeeded in putting together the models of Embden and Meyerhof and filled in some of the crucial details that established the correctness of the scheme. My point here is only to show the fruitfullness of the modeling endeavors of Embden and Meyerhof, not to analyze in detail these subsequent research efforts.

At the time of the publication of Embden's paper, Meyerhof was engaged in a study which indicated that the substance oxidized in the course of reducing pyruvate to lactic acid was still phosphorylated. He proposed that this substance was glycerophosphate. Thus, Meyerhof and McEachern (1933) contains an addendum pointing to the complementary nature of that paper and Embden's scheme. As noted at the end of the last section, Embden's scheme also permitted a simplification of Meyerhof's scheme, for instead of requiring hexosephosphate to lose its phosphate

before scissioning and for a new synthesis of ATP from phosphate and adenylic acid after glycolysis, the phosphate was carried in the intermediaries of glycolysis and could be transferred directly to adenylic acid (or, as we now know, adenosinediphosphate (ADP)). Investigators now turned their attention to determining how the phosphate was transferred to ATP or phosphocreatine. Parnas, Ostern, and Mann (1934) presented evidence that indicated that a substance between phosphoglyceric acid and pyruvic acid was responsible for transferring phosphate to phosphocreatine. They thought this occurred directly but a year later it was established that the phosphate was first transferred to ATP, then to phosphocreatine.

Only one essential part of the overall pathway remained to be filled in. In 1937, Needham, in a theoretical paper reminiscent of the theoretical papers of Embden and Meyerhof, argued for the existence of a second process for esterification of phosphate. Fluoride poisons the formation of phosphopyruvic acid from phosphoglyceric acid, but she noted evidence in both yeast and muscle extract that the esterification of free phosphate still proceeds. She also noted that empirical studies showed more creatine phosphate formed per molecule of lactic acid than the transfer from phosphopyruvic acid to ATP would account for and that the heat output was less than predicted. She therefore proposed that the oxidation-reduction reaction between pyruvate and glyceraldehyde-3-phosphate was accompanied by a second synthesis of ATP. This prediction was confirmed by Needham and Pillai (1937), who established the coupling of the oxidation-reduction reaction and ATP formation. Negelein and Bromel (1939) established that the mechanism for this involved first the formation of a diphosphoglyceric acid in the process of oxidation of glyceraldehyde-3-phosphate followed by a transfer of the phosphate to ATP.

By the end of the decade, thus, a coherent picture of the role of phosphates in glycolysis and in providing energy for muscle action emerged. The process began with the phosphorylation of glucose, partly at the expense of ATP, to hexosediphosphate. This was scissioned into two triosephosphates, which were then further phosphorylated and oxidized. The phosphate bonds in this oxidized product now had a high heat of hydrolysis, which was carried over when the phosphates were transferred to ATP and phosphocreatine. The hydrolysis of these substances provided a source of energy for muscle work. One thus arrives at a picture of the circulation of phosphates which provided a structural integration to the system. (See figure 4 for a linear representation of the overall reactions of glycolysis and an accompanying representation of the circulation of phosphates. The role of NAD^+ and NADH is to transfer the hydrogen removed in the oxidation of triosephosphate to the reaction reducing pyruvate.) Lipmann (1941), introduced the term "high-energy phosphate bond" (symbolized as~P) for those phosphate bonds with a high heat of hydrolysis and in terms of these bonds provided a comprehensive account of the role of phosphates in glycolysis and muscle energetics.

4. REFLECTIONS ON INTERLEVEL THEORIZING

On the basis of this case study of interlevel theorizing, I will now turn to some general issues concerning this kind of endeavor in science. Traditionally, philosophers have empoloyed one framework for understanding connections between theorizing at different levels in science, the model of theory reduction. Causey (1977) provides a clear conception of what such reduction would involve. Causey views nature as consisting of levels, where entities at higher levels are structured wholes

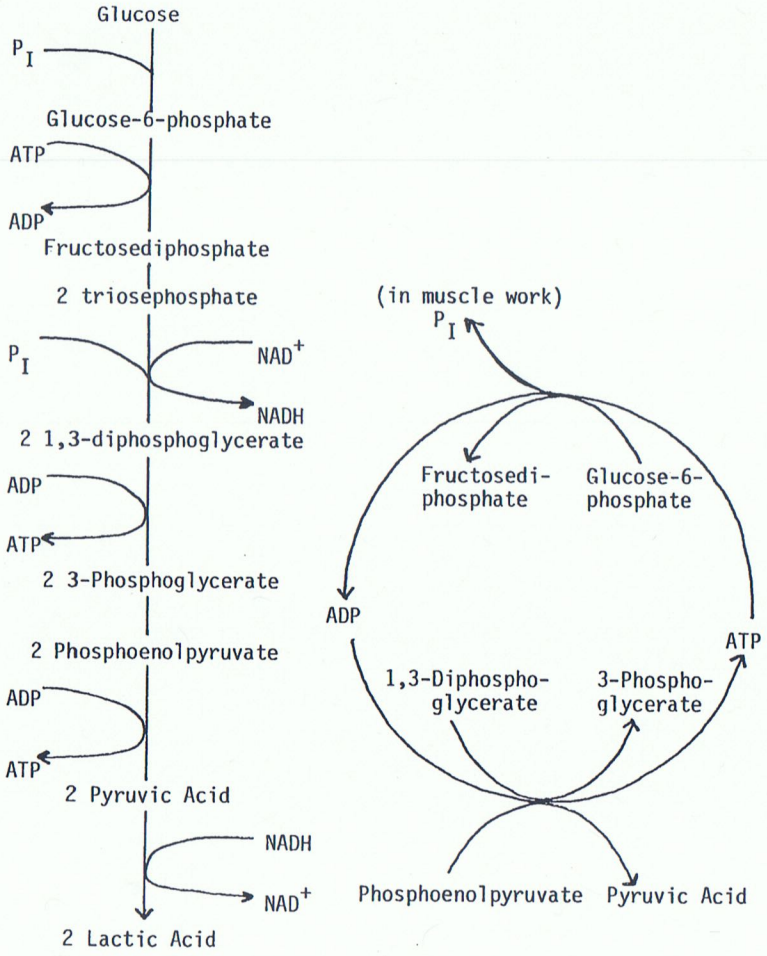

Figure 4: Major reactions on glycolytic pathway (left) and circulation of phosphates (right)

comprised of entities at lower levels. The first task in performing a reduction is to describe structured wholes at one level in terms of their composition from lower level entities so that one can identify the terms referring to objects at the higher level with lower level terms specifying their composition and identify predicates whose extension includes structured wholes with predicates whose extension includes the lower level terms specifying their composition. The second, and more important task, is to derive the law statements about structured wholes, as stated in lower level terms, from law statements about their components and prevailing environmental conditions.

There are serious questions that might be raised as to whether such a program as Causey outlines can meet the objectives he sets for it, increasing explanatory power and providing ontological simplification and unification (see McCauley, 1981, and Bechtel, 1986b). At this point it is enough to note that the program does not account for the case considered in this paper. In bringing together chemical and physiological studies, Embden and Meyerhof did not try to derive physiological theories from chemical ones. Indeed, they were not working with separate chemical and physiological theories; rather, they were trying to fit together information about chemical processes to explain physiological data. Moreover, part of what they discovered - the particular organization of chemical events - was not something that could be derived from their chemical knowledge. It was this organization that is critical to explaining the physiological processes. (To explain this organization in turn, one would have to turn to a quite different kind of theory, evolutionary theory, and explore the character of the interaction of these organized systems with their environment.)

Darden and Maull (1977) introduced the notion of an interfield theory, in which the objective was not to reduce one theory to another, but to build a theory that incorporates principles developed in separate fields of research to solve a common problem. Darden and Maull's notion of a field involves more than a level of organization in nature and includes the scientific pursuits of the investigators. I have not cast my analysis in terms of fields since it is not clear how to differentiate discrete fields in this case. The central investigators were all working on a variety of problems and using numerous techniques simultaneously, creating a complex pattern. However, the notion of an interfield theory as integrating a variety of perspectives of research into a common framework is one that well characterizes the product of Embden and Meyerhof's contributions.

My focus in this paper has been on the research strategies that resulted in the ultimate account of the role of phosphates in glycolysis and muscle energetics. Here it is useful to consider another notion of reduction, one different from the philosophical conception of theory reduction and which captures more of what scientists have in mind by reduction. This notion of reduction is exhibited by what Richardson and I (forthcoming) construe as localizationist research programmes, where the endeavor is to decompose a system into semi-autonomous subsystems (see Simon, 1980, for a discussion of the notion of decomposibility) and to explain the overall operation of the system in terms of these subsystems. Localizationist research has been extremely important to the advance of science and has evolved a powerful set of research heuristics (Wimsatt, 1980). However, it is prone to a variety of errors, involving either mistaken localizations or failing to recognize the importance of systemic organization (see Wimsatt, 1980, and Bechtel, 1982).

In the case I have been analyzing, many of the important contributions were made by researchers pursuing a localizationist endeavor by decomposing the cell into component enzyme processes. However, the contributions of Embden and Meyerhof resulted from their going beyond the results of localizationist research and proposing comprehensive models to try to account for the physiological phenomena. It was only through this model building endeavor that the systemic organization that orchestrates the chemical events came to be recognized. This model building endeavor provided an important complement to the localizationist research, providing an interpretation of the findings of that research (e.g., of Lipmann and Lohmann's difficult to hydrolyze ester and of Lundsgaard's alactacid contraction) and indicating further localized research that was required (e.g., Parnas' investigation as to the point of phosphate transfer).

As I noted at the outset, there is a sense in which during the 1930s biochemists came to rediscover the perspective of the biological tradition that reigned in the late 19th century, for they recognized the cell as an organized system and not as simply a decomposable system. However, there is an important qualification that needs to be added to this remark: the localizationist endeavors during the first three decades of this century were not in vain, for it was they that provided the foundation for the theorizing in which Embden and Meyerhof engaged. The biological tradition did not offer a research strategy of its own. It was the localizationist endeavor that produced the empirical foundations for the new theorizing. In that sense, neither the localizationist endeavor nor the model-building enterprise are self sufficient. Both were needed to figure out the processes of glycolysis and muscular energetics.

A final word is in order about the differences between reductionistic localizationist research and more holistic model-building endeavors. Practitioners of each approach frequently misrepresent the views of those engaged in the other endeavor. This is largely because ultimately both groups accept the same principles: that biological systems, for example, are comprised of specific physico-chemical mechanisms and that these mechanisms are integrated onto a systematic structure. The difference between the two ultimately amounts to one of emphasis, with reductionists focusing more on isolating the mechanisms and the model-builders concerned more with integrating the components to explain higher-level phenomena. In terms of ontology, there is really little difference between their perspectives. However, when one is concerned with the process of scientific discovery, this difference in emphasis becomes important, for it influences the kinds of research that will be undertaken and, in turn, the result that will be produced. Thus, I have tried to show that the model-building emphasis of Embden and Meyerhof yielded a kind of advance that others failed to make, although they, in turn, were dependent on the work of these other investigators.

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