CHAPTER 7

# Integrated Mechanisms

## 1. Introduction: Replacing a Direct Localization

Analysis into localized components and their interactions is a fruitful scientific strategy when the system under study is nearly decomposable; that is, when the organization is relatively simple. In defending *near* decomposability as a heuristic for human problem-solving, Simon offers two markedly different kinds of reasons in its favor. First, given the resource limitations of human beings, near decomposability is an assumption that enables us to deal efficiently with complex systems. This is a kind of naturalistic grounding for simplicity. Second, simply or nearly decomposable systems are more likely to evolve.[1] We have already pointed out that Simon's second reason is less plausible than his first: Evolution works with functioning systems and modifies them to carry out new tasks and meet new demands; it is not creation de novo, but descent with modification. Modifying an existing system may entail altering some of its parts, but this is accomplished by adjusting extant structures to fit new demands. This theory lies at the heart of appeals to evolutionary constraints.[2] As Darwin said, initially appealing to similarities in developmental stages,

> We can clearly understand, on the principle of variations supervening at a rather late embryonic period, and being inherited at a corresponding period, how it is that the embryos of wonderfully different forms should still retain, more or less perfectly, the structure of their common progenitor. . . . Thus we can understand how it has come to pass that man and all other vertebrate animals have been constructed on the same general model. (1871, p. 32)

This development through descent neither depends upon nor creates decomposability. If anything, the mutual coevolution of parts will compromise what decomposability there is and make the system more integrated functionally.

Problems of the sort we will discuss in this and the next chapter present especially difficult problems for localization and decomposition as research strategies because the systems involved exhibit a more complex form of organization. Within some systems, processes depend on the integration of lower-level components, rather than on just their weak interaction. These are *functionally integrated systems*. A research strategy that

decomposes a hierarchically integrated system into units exhibiting independence or quasi-independence will not identify the processes that result from the integration of the system.

Simon characterizes the kinds of nearly decomposable systems with which he is concerned as hierarchical, but he explicitly notes that in so doing he is not requiring that hierarchies incorporate subordination of function. This is one important feature of integrated functions in complex systems: they provide a means by which one component can exercise control over other components. To capture the idea that, in some hierarchies, higher levels exercise control over lower levels, Pattee (1973) introduces a distinction between what he calls *structural hierarchies* and *control hierarchies*. Structural hierarchies are Simon's hierarchical systems. In control hierarchies, demands on systemic function affect constituent behavior, and the mode of organization has the consequence that the interactions between components at one level can alter the behavior of the constituents of these components; that is, processes explicable at a higher level alter processes at lower levels.[3] The result, Pattee claims, is that we can no longer identify a sharp cleavage between levels in nature and thereby develop dynamic theories at one level while ignoring or averaging over processes at others:

> In a control hierarchy the upper level exerts a specific, dynamic constraint on the details of the motion at the lower level, so that the fast dynamics of the lower level cannot simply be averaged out. The collection of subunits that forms the upper level in a structural hierarchy now also acts as a constraint on the motions of selected individual subunits. This amounts to a feedback path between levels. Therefore, the physical behavior of a control hierarchy must take into account at least two levels at a time. (Ibid., p. 77)

Control hierarchies, as far as we understand them, result from the way a system is organized. In functionally integrated systems the behavior of the components is interdependent, so a change in the behavior of one part alters the behavior of others. Thus, the *systems* are self-organizing because of the integration and interdependence of component functions.

As with the cases discussed in the previous chapter, the focal case we consider in this chapter—research on fermentation and muscle glycolysis—began with an assumption of simple decomposability that shifted to near decomposability. Fermentation was assumed to involve a series of discrete steps, each providing products serving as precursors for the next. The research problem was then to identify and localize control for each step. This is complex localization in another domain. We will see that research progressed on the assumption of a linear organization.[4] This is a natural extension of localization and decomposition, acknowledging mini-

mal complexity. Linear organization is less complex, and less demanding cognitively, than other forms of organization that might equally well exist. Simplicity is, thus, a virtue of theories, even if only because of our bounded rationality. Unlike the cases of Chapter 6, though, in these instances the assumption was finally the grounds for its own undoing: after attempting for over three decades to develop a model with linear organization, researchers realized that nature employed a far more complex mode of organization.

There is another important difference between the cases. Research in cognitive neuroscience began with direct localization. More complex models followed only with a shift to lower levels of organization, as a natural consequence of the research program. After initially implicating the hippocampus in memory, O'Keefe and Nadel turned to a yet lower level, asking how the components of the hippocampus realized those higher-level functions and what the intrinsic properties of these components were. In our sense of the term (Chapter 2), these researchers follow a largely "analytic" method. The alternative paradigm to be discussed in this chapter arises when localization turns out to be incorrect; instead of one component controlling an activity, a cadre of components produce it jointly. The problem is then not one of isolating the localized mechanisms, but of exhibiting the organization and the constituent functions. The result is an explanation pitched at the same level as the initial localization; an explanation in terms of organization supplants direct localization.

The research program, accordingly, concentrates on articulating the system's organization. Complex localization requires a functional analysis of system behavior, explaining performance as the product of several different and distinct functional systems. The problem then becomes one of identifying component functions and the underlying organization. It is possible to differentiate component parts of the hippocampus physically and to correlate activity in these components empirically with animal behavior; the physical analysis precedes and aids in the functional decomposition. In the cases we will discuss in this chapter, it was only *after* the complex localizationist explanation had been developed that the structures involved in fermentation were identified physically. Enzymes and coenzymes were identified first in terms of the contributions they made to cellular processes—they were functionally defined as whatever facilitated a certain reaction. It was only later that the precise constitution of enzymes could be determined. Likewise, it was only with the introduction of cell fractionation and the electron microscope in the 1940s and 1950s that researchers were able to identify the sites in the cell where critical enzymes were located and synthesized (cf. Bechtel 1989). As a result, the identification of the steps in the fermentation process, and of the enzymes

and cofactors that figured in that process, was initially given in terms of the functions these agents served.[5]

The experimental strategy for developing the functional decomposition also differed. For the most part researchers had to forgo analytic approaches and pursue synthetic methods. That is, they employed information about the overall reactions and constructed models of how these might proceed by a sequence of basic chemical processes through known chemical intermediaries. Hence, the first step was to construct a model of a possible chemical pathway. This was constrained primarily by knowledge of net reactions and possible chemical reactions, and thus was speculative. Subsequently, as procedures were developed for studying intermediate stages the experimental task was to find empirical evidence relevant to the theoretically driven models.

We will chronicle some critical elements in the history of research on fermentation and glycolysis from the turn of the century through the 1930s in order to show how researchers managed to push beyond a conception of the cell as decomposable or nearly decomposable and came to recognize an integrated mechanism in cell biochemistry. Researchers at the outset of the twentieth century adopted a simple localizationist view, identifying a single unit within the cell as the agent responsible for glycolysis. This will be discussed in section 2. They soon recognized, however, that the process was more complex, that there were several steps in the transformation of glucose into either lactic acid or alcohol. Once it became apparent that direct localization was inadequate, it was still assumed that the cell was a nearly decomposable system in which different enzymes carry out different operations in a step-wise, linear procedure. Each enzyme was thought to be largely or wholly independent of others, and reactions were catabolic: a sequence of enzymes catalyzed a series of reactions, with the output from each reaction providing the input to the next. This attempt at complex localization will occupy us in section 3. As research continued it became increasingly apparent that even these more complex models could not accommodate the evidence produced through experimental procedures and were incompatible with constraints imposed by collateral theories. During the 1930s it was accepted that this conception of metabolism also failed to account for the complex modes of biochemical organization found in the cell, and that it was this organization which allowed the cell to regulate its own processes and serve its physiological functions. These modes of organization were provided by the substances that, in the linear model, were viewed as simply substances entering or leaving the pathway. But many of these substances in fact connect different reactions in the pathway, providing a kind of architecture in which the whole process occurs. This means that one reaction affects other reactions. These linkages are lost when one assumes that the

glycolytic system is nearly decomposable and tries to study individual reactions in isolation. This development of a model with an integrated organization is the emphasis in section 4.

## 2. Direct Localization of Fermentation in Zymase

As we have described the case in Chapter 5, during the nineteenth century there was considerable dispute over whether fermentation was due to chemical agents within living cells, or whether it required the whole living cell. This was a dispute over the appropriate level at which explanation should be pitched. Those, like Liebig, who defended a catalytic account of fermentation were committed to holding that the appropriate level of explanation was chemical. Many researchers contended, however, that some reactions could not be carried out by ordinary chemical catalysts, but instead required the entire cell. Processes conceived at the chemical level were, accordingly, inadequate. It was necessary either to introduce novel agents or to appeal to a higher level of organization. In the wake of Schwann's and Pasteur's work, alcoholic fermentation became the chief example of this class. To mark the distinction between those reactions that depended only on chemical agents and those that seemed to require living cells, the purely chemical agents came to be called *unformed* or *unorganized ferments*, while the living cells came to be known as *formed* or *organized ferments*.[6] Kuhne (1877) introduced the term *enzyme* for unorganized, or purely chemical, ferments (see Teich 1981).

The question whether fermentation could be accomplished by an unorganized catalyst or an enzyme, or whether it required a living cell, was actively debated in the third quarter of the nineteenth century. Several prominent, purely chemical theories of fermentation were advanced by investigators such as Traube (1858), Bertholet (1860), and Hoppe-Seyler (1876). These theories did not gain wide acceptance, in part because researchers were unable to isolate from the cell a catalyst or enzyme that could perform fermentation. If an explanation at the chemical level were correct, then it should have been possible to induce fermentation in the absence of living organisms. This, in turn, would be possible were a catalyst isolated and identified.

One of the first attempts to extract a chemical agent capable of catalyzing fermentation was carried out by Ludersdorff (1846). Ludersdorff ground yeast between glass plates. The resulting paste could *not* perform fermentation. In 1872, Marie Manasseïn performed the same test, but claimed to have more positive results (Manasseïn 1897). However, because it was not clear that all the living yeast cells had been destroyed by her grinding techniques, her results were viewed with some skepticism.

Louis Pasteur also engaged in a grinding experiment and failed to find any evidence of a purely chemical agent (see Kohler 1971). Eduard Buchner's (1897) demonstration that fermentation could be accomplished in a yeast press juice from which all living cells and solid cellular material had been removed was therefore quite a surprise and reversed a long train of negative results.

Buchner initially attributed fermentation to a single enzyme he labeled *zymase*. As Kohler (1973b) argues, Buchner's success provided much of the direction taken by biochemistry in the early decades of the twentieth century. The experimental problem was to isolate and identify enzymes responsible for catalyzing each of the reactions in the cell that did not occur spontaneously in conditions like those found naturally in cells. As Kohler says, this problem virtually defined the research program of biochemistry:

> The new profession of biochemistry that began to emerge about 1900 was initially composed of specialists in a variety of established fields, brought together by a common outlook on the physico-chemical nature of life, a common belief that enzymes were the key agents in the life processes, and shared historical experience, and a new name, biochemistry. (Ibid., p. 181)

Buchner's initial model was committed to simple, or direct, localization. This is somewhat surprising in light of the fact that chemists had by that time accepted fermentation as a complex process involving the scissioning of the sugar molecule and the oxidation of part of the resulting material at the expense of other parts. Organic chemists were already attempting to decompose sugar with alkalies to produce alcohol, and they had successfully isolated several intermediary products. A large part of the explanation for Buchner's adherence to direct localization is that he was simply not part of this research tradition.

Buchner's research was actually part of a tradition focusing on immunology. After the development of the germ theory of disease by Pasteur and Koch, the bacteriological community faced an issue parallel to that which had earlier divided researchers working on fermentation. Some researchers conjectured that the agents of bacterial infection might be the chemical constituents of the bacterial cells, and not the cells themselves. Many of the same researchers pursued the idea that the body's defenses against disease were also chemical in nature.

Hans Buchner, Eduard's brother, figured centrally in this controversy, but took a middle ground between chemists and organicists. He argued that the antibacterial agent was the "living blood plasma," which he took to be a *living* protein (H. Buchner 1889). The idea of living protein was part of a *protoplasm theory* adopted by a number of researchers who tried to integrate chemistry and physiology in the late nineteenth century by

arguing that the basic material of living organisms was chemical in nature, but that this material assumed a special form with special properties in living organisms.[7] Unlike most chemically oriented researchers, who focused on chemical materials as they might be excreted from the cell, Hans Buchner was interested primarily in isolating the proteins that resided *in* the cell.

The project that linked the work of Hans and Eduard Buchner was devoted to grinding bacterial cells to remove the intracellular proteins. To do this Eduard developed a technique for grinding with sand. The debris, however, contained remnants of the cell bodies, and for that reason was regarded as unreliable. It was not until 1896 that one of Hans Buchner's associates, Martin Hahn, developed a technique for filtering the debris to produce the cell juice. This juice rapidly decayed, and it was in the process of trying to find a preservative that sugar was added to it. Eduard Buchner recognized the resulting reaction as fermentation. This was the first clear case of fermentation in a pure cell extract (see Kohler 1971). Because the critical work came out of a project that was principally devoted to isolating bacterial toxins and antitoxins, the interpretation of the work as finding *the* chemical agent responsible for fermentation was not surprising. Within his own problem domain, Buchner's search was clearly directed at whether it was possible to isolate single chemical factors that would produce the pathological reaction. Because the cell extract did produce fermentation, it was natural to conclude that the same agent—whatever it might be—was responsible in intact cells. Moreover, the program of research suggested the agent was a *single* chemical agent.

Eduard Buchner's chemical interests led him to look further at the process by which sugar could be rendered into carbon dioxide and alcohol. By 1904 he provided evidence that "lactic acid plays an important role in the cleavage of sugar and probably appears as an intermediate in alcoholic fermentation" (Buchner and Meisenheimer 1904, pp. 420–21). Eventually Buchner abandoned the simple localization of fermentation in zymase and came to view fermentation as at least a two-step process: Zymase was to be responsible only for the first reaction, producing lactic acid from sugar. The reduction of lactic acid to alcohol was mediated by what he called *lactacidase*.

At the time of the Buchner's success in producing cell-free alcoholic fermentation, the production of lactic acid from sugar in the course of muscle work also was classified as a type of fermentation.[8] The initial linkage of the production of lactic acid with muscle work stemmed from du Bois-Reymond's (1859) discovery of lactic acid in muscles after muscle contraction or the death of the animal. Hermann (1867) inferred that this reaction was anaerobic, as is alcoholic fermentation, and Bernard showed that it was a general reaction in animal tissue:

> This lactic ferment occurs in the blood, in the muscles, even in the liver, since I have found that muscle and animal tissues do not become acid after death unless they contain sugar or glycogen which rapidly undergoes a lactic fermentation. (1877a, p. 328)

The final convincing demonstration of the linkage between lactic fermentation and muscle work was found in the research of Fletcher and Hopkins (1907). As we shall see, the relation between alcoholic fermentation and lactic acid fermentation was crucial in unraveling the mechanism of fermentation. Eventually biochemists came to use *glycolysis* to refer generically to both processes.

## 3. A Complex Linear Model of Fermentation

The demonstration that fermentation could be carried out independently of living cells reawakened interest in developing a chemical account of fermentation. The task of determining the sequence of reactions fell to biochemists during the first three decades of this century and culminated in the basic understanding of the chemical stages in fermentation developed in the 1930s. Biochemistry placed several constraints on an acceptable solution to the problem: the first is an operational constraint, requiring independent evidence for the processes involved; the second located the processes at the chemical level; and the third is tantamount to near decomposability. Let us look briefly at each and then turn to consider their impact in more detail.

The requirement of independent evidence for postulated processes is particularly challenging in the case of reactions within living systems, because it is difficult to gain direct access to the reactions. As in many other cases, researchers of fermentation had to rely on indirect evidence. It was assumed that fermentation began with the scissioning of the sugar molecule into three-carbon sugars and ended with the decarboxylation of a three-carbon compound to form alcohol. The problem was in determining the intervening reactions. It was not enough to produce a chemical model of *possible* intermediaries; it was necessary to determine the pathway *actually used* in living organisms. One way to demonstrate the correctness of a model pathway was to show that the proposed intermediaries actually did arise in the process of fermentation.

One source of evidence for the occurrence of a proposed intermediary would be the discovery of a small quantity of a substance among the reaction products. This proved to be difficult technically, mainly because metabolic reactions tend to occur rapidly, with the intermediate products from one reaction being consumed almost immediately in subsequent reactions. Another, more useful source of evidence was an increase in con-

centrations of a potential intermediate when the reaction was interrupted in some way. In classical inhibition studies of this type, a variety of substances were used to poison the cell, stopping the reaction at a specific stage and bringing about the buildup of intermediate products. Evidence that a potential intermediary could be metabolized by the living tissue in question served as a third source of information. One of the principal tests used to determine which of the intermediaries actually figured in fermentation was to insert the intermediary into cells, or (more often) cell extracts, to determine whether it was consumed in the reaction. Researchers generally described these three procedures as tests of whether the intermediary would itself "ferment."[9] It was important not only that the potential intermediary ferment, but that its rate of fermentation be at least as rapid as that of the sugar itself. Failure to ferment at an acceptable rate would count against the substance being an actual intermediary in fermentation and, accordingly, against the model.

The second constraint on an acceptable explanation of fermentation was that *each* stage in the process must involve a known, basic chemical reaction. From a chemical perspective, it had long been clear that fermentation was a complex process. As organic chemists understood reactions toward the end of the nineteenth century, they held that basic chemical reactions involved the addition or removal of small chemical groups (see Table 7.1). These constituted the reactions nineteenth-century biochemists were prepared to accept in models in which fermentation was regarded as a chemical process. The fact that fermentation was obviously not such a simple chemical reaction, but was far more complex, placed it outside the domain of basic chemistry. Those researchers with a chemical orientation recognized the need to break the reaction into a sequence of reactions, where the component reactions would be the required, basic chemical reactions.

The third constraint was the assumption that the overall reaction involved a linear degrading of the initial substrate to produce the output. This assumption was tantamount to near decomposability because, if it held, each reaction necessarily occurred in relative independence and

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| Oxidation | The addition of an oxygen molecule. |
| Reduction | The addition of a hydrogen molecule. |
| Dehydration | The removal of a water molecule from substrates. |
| Decarboxylation | The removal of a carbon dioxide molecule. |
| Deamination | The removal of an ammonia molecule. |

Table 7.1. Basic Chemical Reactions. These were some of the allowed chemical reactions as understood by organic chemists toward the end of the nineteenth century.

could be studied in isolation, provided the appropriate inputs were available. The idea that fermentation would be linear was natural at the time. On the one hand, the product of fermentation—alcohol—was far simpler in structure than sugar. On the other hand, the overall reaction released energy. Research then progressed on the assumption that fermentation was a sequence of catabolic reactions, with each step yielding a simpler intermediary and releasing energy until alcohol was produced.[10]

While there was explicit reference to the first two constraints, this last one was generally left implicit. Knoop (1904) came close to an explicit statement of it, though, after proposing the model of $\beta$-oxidation for fatty acids. He said the goal was "a knowledge of the course of the decompositions and oxidations in the building materials and nutritional substance of the animal organism that would leave no gaps" (ibid., p. 3). By referring only to decompositions and oxidations, Knoop left no place for syntheses and reductions, and hence appeared to be calling for a linear model. In discussing the linearity of metabolic mechanisms, Thunberg was more explicit:

> I consider that nothing of the oxygen consumed in the general metabolism is found in the expired carbon dioxide. I consider the catabolism of the food stuffs to take place in a series of continuous dehydrogenations, carried out by a series of dehydrogenases. This procedure, to which the complicated food-stuff molecules are thus subjected, might be compared to what happens in modern factories where a piece of metal glides along on rails from workman to workman each of whom has his special task to carry out in the course of the work until the metal piece leaves their hands as a finished product. (1930, p. 327)

In one sense linearity may have represented an attempt to start with the simplest model and to introduce complications only as needed. However, a linear model was in fact a logical presupposition of the requirement that proposed intermediaries ferment as rapidly as sugar. If the pathway were not linear, then skipping an earlier step might impede later steps and so undercut the assumption that the reaction of an intermediary could proceed without starting at the beginning of the reaction pathway. Linearity was deeply ingrained in the biochemical research program.

Each of these assumptions can be more fully illustrated in work within the biochemistry of the period. We will take them up in turn and consider their implications.

### *The Search for Intermediates*

After Buchner's success in cell-free fermentation, investigators began to search for evidence as to which organic substances might figure in biological fermentation. As we have noted, Buchner proposed a two-step

process when he modified his initial, simple localizationist account of fermentation, using lactic acid as an intermediary. Not only had lactic acid been generated as an intermediary in attempts to degrade sugar with alkalies, but Buchner and Meisenheimer (1904) claimed to have found small amounts of lactic acid as products in alcoholic fermentation. Lactic acid was, however, soon rejected as an intermediary by most researchers. The crucial result was Slator's (1906) demonstration that lactic acid could *not* be fermented at all rapidly by yeast.[11] He concluded on that basis that it did not figure importantly in the pathway.

An independent line of research provided evidence of other intermediaries. In the late nineteenth century, organic chemists had carried out a number of experiments, treating glucose with different alkalies. Strong alkalies such as caustic potash resulted in the production of alcohol and carbon dioxide, while weaker alkalies produced lactic acid (Duclaux 1896). Further research in this vein suggested that several three-carbon compounds, including methylglyoxal, glyceraldehyde, and dihydroxyacetone, might be intermediaries in the process (for an overview, see Harden 1932). Attention then turned to these three-carbon substances and, in particular, to whether these substances would ferment as rapidly as sugar. Some researchers, including Buchner, found evidence that methylglyoxal was not able to be fermented by yeast. Nonetheless, as we shall see, it figured centrally in one of the first comprehensive models of fermentation. Evidence was also presented showing that dihydroxyacetone and glyceraldehyde did ferment in living yeast, although the evidence was controversial.

Otto Neubauer's research on amino acid metabolism drew attention to pyruvic acid as another potential intermediary. Neubauer and Fromherz were investigating the oxidative deamination of alanine in yeast and showed that the reaction did not stop with pyruvic acid, but yielded alcohol. Having established the role of pyruvic acid as an intermediary in alanine metabolism, Neubauer proposed that it might also play a role in sugar fermentation. He said pyruvic acid

> could be an intermediate in the alcoholic fermentation of sugar. . . . We ask colleagues to leave to us the further study of the role of pyruvic acid in the fermentation of sugar; also, it is intended to study the question whether it is an intermediate in the combustion of sugar in the higher animal organism. (Neubauer and Fromherz 1911, p. 350)

Evidence rapidly accumulated supporting pyruvic acid as an intermediary. Neubauer demonstrated that it could be fermented, and Carl Neuberg demonstrated the same for pyruvate. Neuberg also identified an enzyme, carboxylase, which catalyzed the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. And Fernbach and Schoen (1913) finally

isolated a calcium salt of pyruvic acid in the products of fermentation carried out in the presence of calcium carbonate.

Evidence for the occurrence, or at least the potential reactivity, of intermediates thus played an important operational role. In the case of Buchner, the observation that lactic acid could not be fermented naturally served as a reason for rejecting his proposed model. In the case of Neuberg, the ability to naturally degrade pyruvic acid provided a defense for the feasibility of his model. As we shall see, however, this evidence concerning the existence and reactivity of intermediaries provided only a relatively weak constraint on the acceptability of a model of fermentation.

### *The Appeal to Basic Chemical Reactions*

The research on the decomposition of sugars by alkalies resulted in numerous models in which basic chemical reactions accounted for each proposed stepwise change. These models, in turn, provided inspiration for developing models of biological fermentation. Although a number of proposals were introduced in the first decade of the twentieth century, the first to command wide acceptance was developed by Carl Neuberg (Neuberg and Kerb 1914). Neuberg thought the glucose molecule was separated into two molecules (see Figure 7.1) of methylglyoxal (step 1), one of which was reduced to glycerol, yielding pyruvic acid as a byproduct (step 2a). The pyruvic acid was then decarboxylated, yielding acetaldehyde and carbon dioxide (step 3). The acetaldehyde produced in this step participated in a subsequent oxidation, being reduced to alcohol while another molecule of methylglyoxal was oxidized to pyruvic acid. After the initial release of pyruvic acid, step 2a was unnecessary, with the reaction proceeding through step 2b. Neuberg's model was widely accepted during the next twenty years. It had many virtues. Besides providing a sequence of reactions that produced alcohol from sugar through simple known reac-

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| (1) | $C_6H_{12}O_6$ [Hexose] | $\Rightarrow$ | $2\,C_3H_4O_2$ [Methylglyoxal | + | $2H_2O$ Water] |
| (2a) | $2C_3H_4O_2 + 2H_2O$ [Methylglyoxal + Water] | $\Rightarrow$ | $C_3H_8O_3$ [glycerol | + | $C_3H_4O_3$ pyruvic acid] |
| (2b) | $C_3H_4O_2 + C_2H_4O + H_2O$ [Methylglyoxal + Aldehyde + Water] | $\Rightarrow$ | $C_3H_4O_3$ [Pyruvic Acid | + | $C_2H_5OH$ Alcohol] |
| (3) | $C_3H_4O_3$ [Pyruvic Acid] | $\Rightarrow$ | $C_2H_4O$ [Acetaldehyde | + | $CO_2$ Carbon Dioxide] |

Figure 7.1. Glycolytic Pathway Proposed by Neuberg and Kerb (1914). Neuberg and Kerb developed a simple model explaining the breakdown of glucose into alcohol in fermentation.

tions, his model could account for the methyl that had been found among the products of fermentation.[12]

There were also important objections to Neuberg's model, two of which are of particular note for our purposes. The first concerned the role of phosphates in fermentation. Although Buchner showed that fermentation could occur in cell-free extracts, it occurred much more slowly than in living cells. Moreover, after a short period the reaction almost totally stopped. Arthur Harden (1903) established that adding blood serum would yield an 80% increase in fermentation. In subsequent collaborative work with William Young (Harden and Young 1906), Harden showed that adding a phosphate could maintain the reaction. Harden and Young (1908) proposed that this phosphate, which appeared to be consumed in the reaction, was taken up into an ester—hexosediphosphate—with one molecule of the sugar, while a second molecule was fermented. The resulting hexosediphosphate slowly decomposed in a hydrolytic reaction, liberating sugar and phosphate which could again enter the glycolytic reaction (see Figure 7.2). What is especially noteworthy is that Neuberg's model did not provide a role for phosphates, though they did appear to be integral to the process. Several researchers faulted the model on this point, but Neuberg dismissed the evidence for incorporating phosphates, as well as for the production and accumulation of hexosediphosphate, in yeast-juice preparations as an artifact of the experimental design. He contended flatly that phosphates could not figure in fermentation in living cells, because hexosediphosphate was not fermented in living cells (Neuberg and Kobel 1925).

Neuberg's objection to phosphate models rested on the principle that nothing could be an intermediary in fermentation that could not be fermented *as quickly as* the sugar substrate—a principle that seemed entirely reasonable on the surface. If we assume that the overall process is nearly decomposable into its component steps, each of which can be performed in isolation, then a substance that cannot undergo the necessary reaction cannot be an intermediary. Given this much, the rejection of

**Glycolytic Reaction**:

$$\underset{\text{[Hexose + phosphate]}}{2C_6H_{12}O_6 + 2\,R_2HPO_4} \Rightarrow \underset{\text{[Hexosediphosphate + Alcohol]}}{C_6H_{10}O_4(PO_4R_2)_2 + 2C_2H_5OH + 2CO_2 + 2H_2O}$$

**Hydrolytic Reaction**:

$$\underset{\text{[Hexosediphosphate]}}{C_6H_{10}O_4(PO_4R_2)_2 + H_2O} \Rightarrow \underset{\text{[Hexose + Phosphate]}}{2C_6H_{12}O_6 + 2R_2HPO_4}$$

Figure 7.2. Harden and Young's (1906) Conception of the Role of Phosphates in Glycolysis. An alternative model of glycolysis, depending critically on phosphates.

hexosediphosphate as an intermediary in fermentation seems quite sensible.

Ironically, Neuberg's own model was subject to criticism on this same point. Despite numerous attempts, researchers could not show that methylglyoxal could be fermented in living yeast. In this case, however, Neuberg responded quite differently, proposing ad hoc explanations for this failure. He suggested, for example, that the added methylglyoxal might have a different structure than that produced naturally in the reaction and that it might not be able to reach the necessary reaction site within the cell.

### *The Assumption of Linearity*

We have already seen that linearity had an effect on the work on fermentation. The hypothesis that intermediaries must ferment as rapidly as sugar itself assumes that later stages in the reaction are dependent on earlier stages only for their products. In fact, the one intermediary considered that actually does figure in fermentation was ruled out on just this ground: hexosediphosphate accumulated in the course of fermentation in cell extracts in part because reactions later in the normal process were cut short. The result was that reactions earlier in the pathway were also halted.

We have noted that linearity is a natural simplifying assumption. Chains of reactions are more readily tracked and understood than ones involving cycles. However, like other constraints, this assumption can be abandoned when other considerations tell against it, or when it conflicts with the other constraints we have mentioned. This can be seen in failures of linearity in some early models, such as Neuberg's. The final product of the reaction in Neuberg's model, ethyl alcohol, was in a lower oxidation state—that is, it had a higher energy level—than pyruvic acid, which was supposed to be an intermediate product in the reaction. This meant that the formation of pyruvic acid required an endothermic reaction and thus violated the assumption that the process involved simply a sequence of catabolic reactions. Moreover, Neuberg's model assumed that the aldehyde formed from pyruvic acid figured in an *earlier* reaction in which it was reduced at the expense of methylglyoxal (and which was in turn oxidized to yield more pyruvic acid). Effectively, a feedback loop was being inserted into the overall pathway. Not only was step 3 dependent on step 2b, but 2b was dependent on 3. The feedback loop was included because the alternate course, represented by step 2a, would lead to a buildup of glycerol. Glycerol was not found in normal cells, and no alternative set of reactions could be conceived through which the glycerol would be broken down. Our acceptance of this willingness to compromise linearity is no more than a recognition that problem solving is a process of simultaneous constraint satisfaction.

## 4. An Integrated System Responsible for Fermentation

Gustav Embden and Otto Meyerhof were pivotal in overcoming the assumption of linearity and establishing the integrated character of cellular fermentation. Neither initially rejected a linear organization, but the synthetic character of their approaches eventually led them to develop nonlinear models. One distinctive feature of their methodology was that they did not limit their focus to the basic chemical information and biochemical modes of experimentation. Each conceived of the problems physiologically, and therefore functionally, and each developed models of the physiological role of fermentation. As a result they looked at fermentation in the broader context of cell life and not the narrower context of an isolated biochemical problem. They fostered the development of models that more obviously violated the assumption of linearity and which therefore had the effect of undermining near decomposability.

### *Embden and Meyerhof: The Role of Phosphates*

Embden introduced the idea of phosphorylated intermediaries in the course of research into parallels between alcoholic fermentation and lactic acid fermentation in muscle glycolysis. This in turn was part of an attempt to explain how lactic acid fermentation figured in producing energy for muscle contraction. Embden and his collaborators attempted to simulate Buchner's work with alcoholic fermentation by carrying out lactic acid fermentation in muscle extract. They established that lactic acid increased in the muscle extract over time, but noted that adding glucose failed to increase the yield (Embden, Kalberlah, and Engel 1912). Embden hypothesized that the lactic acid was derived not directly from glucose but from an unknown precursor which he called *lactacidogen*. Embden, noting that lactic and phosphoric acids were produced in his extract in equimolar proportions, concluded that lactacidogen was the precursor of both substances. Because lactacidogen was likely to be a phosphorylated sugar, Embden decided to test the hexosediphosphate ester of Harden and Young by adding it to his muscle extract. That too increased the production of lactic acid. As a result, Embden proposed that it might be identified with lactacidogen (Embden, Griesbach, and Schmitz 1914).

Meyerhof also considered the possibility that phosphorylated substances might be intermediaries in fermentation. In 1924 he offered evidence that phosphorylated hexosediphosphate split into triose diphosphates without first undergoing hydrolysis as Harden and Young proposed. Subsequently he provided further evidence for the role of phosphates by using fluoride to inhibit glycolysis. This yielded a buildup of hexosediphosphate and indicated a more active role for this substance

than had been envisioned by Harden and Young. Meyerhof, however, confronted the same obstacle others faced: hexosediphosphate could not be fermented in artificial solutions. In response he proposed that a special, active form of hexosephosphate served as an intermediary. One molecule of this 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 subsequently split into two monophosphates that could reenter the pathway.[13]

In the early 1930s Embden took a step beyond Meyerhof, proposing a model in which phosphorylated substances were the precursors of lactic acid. According to this model (Figure 7.3), hexosediphosphate is scissioned into two triosephosphates. One of these triosephosphates is oxidized to 3-phosphoglyceric acid while the other is reduced to glycerophosphoric acid. The 3-phosphoglyceric acid is next dephosphorylated to pyruvic acid and then reduced to lactic acid. At the same time, the glycerophosphoric acid is oxidized to form triosephosphate (Embden, Deuticke, and Kraft 1933). Embden's model was not entirely a speculative proposal designed to allow for a phosphorylated precursor to lactic acid. There was empirical evidence for the critical first step in earlier work by Lipmann and Lohmann (1930). However, when Embden published his scheme the proof for many of its parts was not yet developed. The model eventually proved incorrect in detail, but the development of a model using phosphorylated intermediaries to account for physiological phenomena was a critical ingredient in ultimately coming up with more adequate theories. Embden's awareness that muscle contraction produced free phosphate put him on the path to a radically different understanding of fermentation and facilitated the discovery that phosphate bonds provided the mechanism by which the energy released in the metabolic reactions was transferred to points where it was used.

Acceptance of the importance of phosphate bonds to fermentation was somewhat impeded by the assumption that fermentation must be a series of catabolic reactions; that is, by the assumption of linearity. As a result of the work of Fletcher and Hopkins (1907) it had been generally assumed that the energy liberated in lactic acid formation was the direct source of the energy for muscle contraction, with the energy being transferred in the form of heat. Fletcher and Hopkins's investigations inspired a number of detailed quantitative studies of the relationship between glucose levels, lactic acid levels, and the response of each to oxygen. These studies were coupled with investigations, carried out principally by Hill and Meyerhof, into the relation between the chemical events in the cell and the heat production. Three important results emerged that were regarded as anomalous on the assumption that lactic acid formation was the direct source of energy for muscle contraction. First, heat production was

| | | |
|---|---|---|
| $C_6H_{10}O_4(PO_4H_2)_2$ [hexosediphosphate] | ⇒ | $2C_3H_5O_2(PO_4H_2)$ [triosephosphate] |
| $2C_3H_5O_2(PO_4H_2) + H_2O$ [triosephosphate] | ⇒ | $C_3H_5O_3(PO_4H_2) + C_3H_7O_2(PO_4H_2)$ [3-phosphoglyceric acid + glycerophosphate] |
| $C_3H_5O_3(PO_4H_2)$ [3-phosphoglyceric acid] | ⇒ | $C_3H_4O_3 + H_3PO_4$ [pyruvic acid + phosphate] |
| $C_3H_4O_3 + C_3H_7O_2(PO_4H_2)$ [pyruvic acid + glycerophosphate] | ⇒ | $C_3H_6O_3 + C_3H_5O_2(PO_4H_2)$ [lactic acid + trisephosphate] |

Figure 7.3. Glycolytic Pathway Proposed by Embden, Deuticke, and Kraft (1933). This model incorporated phospholated intermediaries as precursors to lactic acid.

greater during oxidative recovery (when lactic acid was metabolized) than during anaerobic contraction (when lactic acid was formed). This was puzzling because the greatest energy expenditure was during the anaerobic phase. Second, the heat produced during contraction was greater than that theoretically available in the production of lactic acid from glycogen. And third, the heat produced during recovery was less than would be expected on the basis of complete oxidation of lactic acid. These discrepancies made it difficult to account for the energy of muscle contraction directly in terms of the thermodynamics of metabolism (see Needham 1971).

Meyerhof's proposal of a lactic acid *cycle* provided the first apparently adequate explanation of these results. Whereas earlier accounts construed all the lactic acid as being oxidized during recovery, Meyerhof (1924) proposed that approximately three-fourths of the lactic acid produced in anaerobic contraction was reconstituted as glycogen during recovery. He postulated that the energy for resynthesis came from coupling the resynthesis with the oxidation of the smaller portion of lactic acid. The fact that not all lactic acid was oxidized would explain why the heat production in this phase was less than expected.

The incorporation of this cycle into Meyerhof's model was important for a number of reasons. It represents one of the earlier cases in which a more complex mode of organization was posited, rather than a simple catabolic process (Nachmansohn 1972, p. 5). It was also important that a coupling of reactions—a nonlinear dependence—was postulated simply to handle the thermodynamics, even though Meyerhof lacked any knowledge of the mechanism for coupling. The constraints that led to a more complex organization were top-down rather than bottom-up. The lactic acid cycle, however, could not account for the additional heat produced during anaerobic contraction. Fitting the chemical events with the thermodynamic data required the recognition that the energy for muscle contraction did not come directly from glycolysis. Embden offered evidence as early as

1924 that not all the lactic acid formation occurred in the course of muscle contraction, but making systematic sense of this depended on finding some other source for the energy of contraction. The solution was found only with the identification of the function of two other compounds discovered in the late 1920s. Eggleton and Eggleton (1927) isolated a rapidly hydrolyzable substance from the cell that released large quantities of phosphorus. At the time this substance was called *phosphagen*, but is now known as *phosphocreatine*. Lohmann (1929) and Fiske and Subbarow (1929) isolated another compound that was readily hydrolyzed into adenylic acid and pyrophosphate. This substance, first named *andenylphyrophosphate*, subsequently came to be known as *adenosine triphosphate* or ATP.

### *The ATP/ADP Cycle*

Once ATP and phosphocreatine were chemically identified as cellular substances, the observation was made that both of these, upon hydrolysis, released large quantities of heat (Meyerhof and Lohmann 1927). The recognition that these compounds were indeed the proximate agents of energy for muscle contraction and the way in which the compounds functioned resulted from an inhibitory study by Lundsgaard (1932). He injected rabbits with iodoacetate and discovered that it was highly toxic. The pattern of this toxicity was important. With exercise the animal entered rigor mortis without the formation of lactic acid. Lundsgaard also showed in extract from frog muscles poisoned with iodoacetate that muscular contractions prior to the onset of rigor mortis occurred without the formation of lactic acid, but with the breakdown of phosphocreatine. This established that phosphocreatine could provide the energy for muscular contraction and suggested that it was also the source of energy in normal muscle.

The scene was now set for Meyerhof and his coworkers to bring ATP into the account of glycolysis and to piece together a picture of the previously mysterious role of phosphate bonds in glycolysis. Lohmann (1931) established that ATP was necessary for fermentation, and, together with Meyerhof, he suggested that ATP served as a link between glycolysis and the resynthesis of phosphocreatine:

> The adenylpyrophosphate [or ATP] cycle maintains the lactic acid formation. The synthesis of phosphagen [or phosphocreatine] is therefore made possible . . . by the cleavage energy of the adenylpyrophosphate, while the energy of lactic acid formation (from phosphate esters) serves to resynthesize the cleaved pyrophosphate. (Meyerhof and Lohmann 1932, p. 576)

Lactic acid fermentation was linked to other processes through which ATP and phosphocreatine were synthesized and broken down. It was no longer viewed as a discrete process.

Meyerhof, Lohmann, and Meyer (1931) recognized that ATP was a critical component in the coenzymes of both alcoholic and lactic acid fermentation. The need for a coenzyme had previously been seen in the same investigations of Hardin and Young that showed the need for a phosphate. Since the coenzyme was needed for the early steps of glycolysis, this suggested that ATP figured there, too (it contributes one of the phosphate bonds in the formation of hexosediphosphate). Meyerhof and Lohmann (1932) established, further, 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. (Ibid., p. 460)

This research made it clear that ATP served an important function in integrating biochemical processes. Further investigations by Lohmann established that ATP also functioned in the hydrolysis of creatine phosphate during muscle action. He also suggested that ATP regulated glycolysis, insuring that it occurred when needed to replenish energy used:

> Viewed teleologically, this dual function seems to be a very ingenious arrangement for insuring the orderly sequence of the chemical processes involved in the muscle twitch. . . . The contraction brings about a fission of adenylpyrophosphoric acid which in turn imposes a cleavage of creatine phosphate, thereby simultaneously reconstituting adenylpyrophosphoric acid; the latter can now interact as co-enzyme by mobilizing glycogen for lactic acid formation. (Lohmann 1934/1969, p. 60)

Another crucial link in the process of ATP formation was recognized in 1937, when Needham, in a theoretical paper reminiscent of those of Embden and Meyerhof, argued for the existence of a second process for

esterification of phosphate. She noted that fluoride would stop ATP formation, given the Meyerhof and Lohmann model. She continued to point out that in both yeast and muscle extract poisoned with floride, some ATP does form. Needham also argued that empirical studies showed (1) that more creatine phosphate formed per molecule of lactic acid than the transfer from phosphopyruvic acid to ATP would account for and (2) that the heat output was less than predicted. She therefore proposed that the reaction 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.

By the end of the decade a coherent picture emerged of the role of phosphates in glycolysis and in providing energy for muscle action. Glycolysis began with the phosphorylation of glucose, partly at the expense of ATP, to form 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.

The circulation of phosphates provides a biochemical integration to the system. Whereas glycolysis has previously been assumed to be a linear and nearly decomposable process, with a series of discrete catabolic reactions, the model that emerged in the 1930s revealed the system as highly integrated. If we focus on the breakdown of sugar to alcohol or lactic acid as central, the process can be represented as a linear pathway (see Figure 7.4). The coenzymes that link cellular reactions are simply shown as byproducts peripheral to the main process. This ignores the role of these coenzymes in integrating cellular reactions. These substances—ATP, ADP, NADH, and $NAD^+$—function as links in a complex process that is more perspicuously represented as cyclic (Figure 7.5). What constitutes a product of one reaction is an input to other reactions that occur earlier in the breakdown of glucose to lactic acid or alcohol. These linkages make the fermentation system one that cannot be simply decomposed into its component reactions without seriously misrepresenting the processes occurring in the cell[14] (for further development of this case, see Bechtel 1986b).

## 5. Conclusion: The Discovery of Integration

We have focused on the process by which biochemists came to unravel the mechanism underlying fermentation. Buchner, in the wake of his discovery of cell-free fermentation, defended direct localization, assigning the reaction to a single enzyme which he labeled zymase. Had it turned out

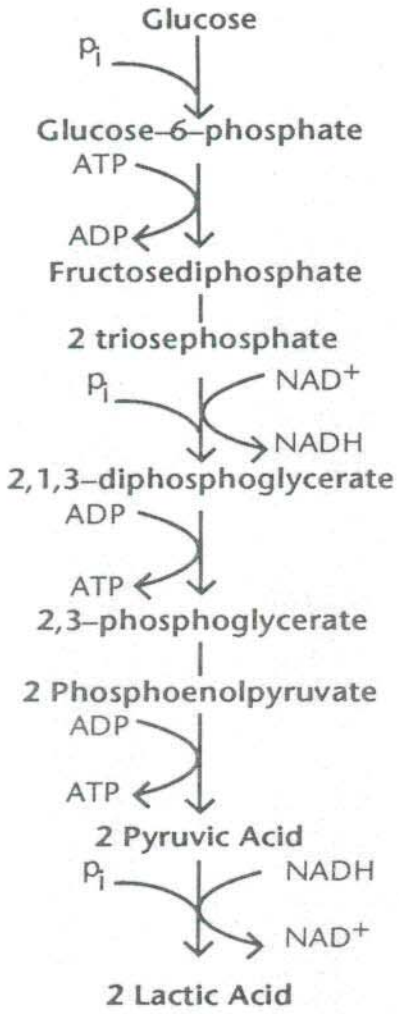


Figure 7.4. A Standard Representation of Glycolysis as a Linear Process. The breakdown of glucose is represented as a linear process with a variety of intermediaries and several byproducts, including ADP, ATP, NADH, and $NAD^+$.

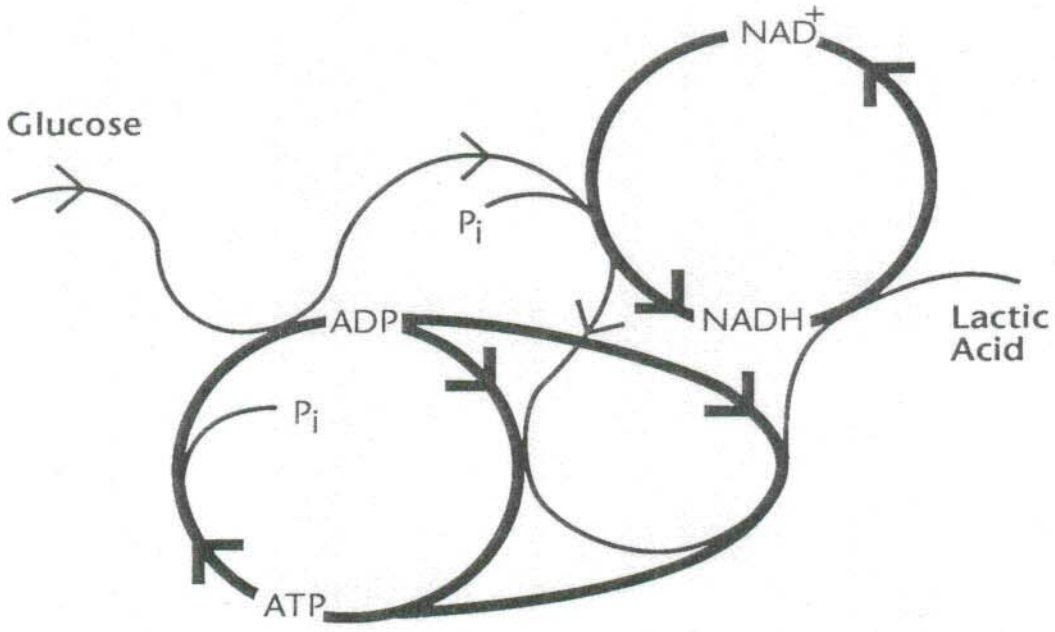


Figure 7.5. An Alternative Representation of Glycolysis as Cyclic. This alternative (and somewhat simplified) representation acknowledges the critical role of the ATP-ADP cycle and the NADH-$NAD^+$ conversions in glycolysis. Linearity is explicitly compromised.

that one enzyme was responsible for fermentation, research should then have turned to a lower level to explain how this enzyme functioned. In this case, however, direct localization was flawed. Fermentation is not a simple chemical reaction; a number of enzymes and coenzymes figure in the process. An explanation at the same level as the incorrect direct localization was required to understand fermentation.[15] We have identified a variety of constraints on the emerging explanation. It was, at least initially, assumed that the overall process would be decomposable into a linear sequence of reactions. The product of one reaction would serve as the substrate for the next, and the ordering would be provided by the energy available in the compounds. This assumption was coupled with the hypothesis that any intermediary in the process must ferment as rapidly as the sugar itself. These assumptions led researchers to dismiss phosphorylated substances from the pathway for nearly twenty years and to search instead for a means by which methylglyoxal could be fermented. Ultimately it was discovered that the fermentation system was minimally decomposable. Various reactions were mutually interdependent. If we experimentally disrupted these reactions, then even a normally fermentable substance would not ferment. Even more significantly, disrupting one step in the pathway would block others that preceded it. We now think of the breakdown of ATP to ADP as the critical reaction, since it releases energy for work. However, disrupting processes that would otherwise appear obviously downstream (for example, the formation of pyruvic acid) will inhibit the formation of ATP. Isolating the process of glycolysis from that in which ATP is broken down to ADP also prevents glycolysis, as some of the reactions in glycolysis require ADP. Interaction and organization in the system are critical.

Discovering the organized context within the cell permitted researchers to overcome the long-standing debates over whether processes in the living cell are common chemical effects or of some different nature. The apparent differences between ordinary chemical reactions and those in living systems are the result of the organization found in the cellular environment. The basic reactions are chemical, and organization serves to modulate their operation. Rudolf Peters (1957) offers the following retrospective account of the transformation of biochemistry as it came to deal successfully with organization:

> We seem to have travelled very far from the old controversies, now hardly embers even. That of vitalism versus mechanism seems to me particularly dead at the moment, because most of us, I imagine, now feel that the one thing which distinguishes the living system is that complexity of its organisation, and with the increasing interest in nucleic acid chemistry, some of us may believe that if this system is put together it will work. (P. 372)

The attempt to decompose the cell and study the reactions in isolation did not directly reveal the mechanisms critical to fermentation. Moreover, the research led to systematic errors in the understanding of fermentation, including the exclusion of phosphates from the main fermentation process. These errors were overcome only by researchers whose focus was more directed at the physiological needs of the cell, and evidence about how whole cells behave. Thus Embden saw that lactic acid fermentation in muscle juice did not use straight glucose, but required an intermediate form. He also saw that inorganic phosphate was produced in fermentation, and he inferred that the intermediaries probably were phosphorylated. Meyerhof developed models of the physiological processes in which the chemical mechanisms underlying the process could not yet be specified.[16] From the perspective of developing reasonably simple models, phosphorylated intermediaries seemed extraneous and unnecessary. They certainly compromised linearity. In the end researchers discovered that the system responsible for fermentation was *not* nearly decomposable: interaction and organization were critical to the process.

This research program is thus quite different from what we saw in Chapter 6. Instead of finding a component, or a center, exercising an autonomous function, and then proceeding to a lower level to explain its operation, researchers here stayed at essentially the same level and sought to identify the array of components involved in fermentation and to understand their organization. This is what we call *first order interaction*. Instead of isolating independent "organs" whose behavior is relatively independent, first-order effects require an understanding of organization and interaction. This is another alternative, in addition to first order independence, represented in Figure 7.6. The central tasks involve isolating components, their interactions, and the organization that makes those interactions possible. Thus, in explaining fermentation, we eventually must turn to the specific reactions, the enzymes that facilitate them, and the interactions between various stages in the process. This is essentially a functional analysis of the system.

What is critical in understanding an integrated system is the willingness to go beyond what can be learned by studying component reactions in isolation. One must attempt to develop comprehensive models that integrate different processes in accounting for systemic behavior. Such synthetic approaches are obviously fallible. Gustaf Embden, for example, was considered by some of his contemporaries to be excessively speculative. An analytical method, though, is also fallible. Indeed, as we have seen in assuming decomposability or near decomposability, the analytic method may lead us to miss critical interactions. There is no guarantee that the processes are as independent as the analytic method assumes, and as a

result it is possible to overlook important features of the system by insisting on an analytical approach. Integrated systems may not escape our observations and understanding altogether, but they may require that we finally accept a failure of decomposition and localization.

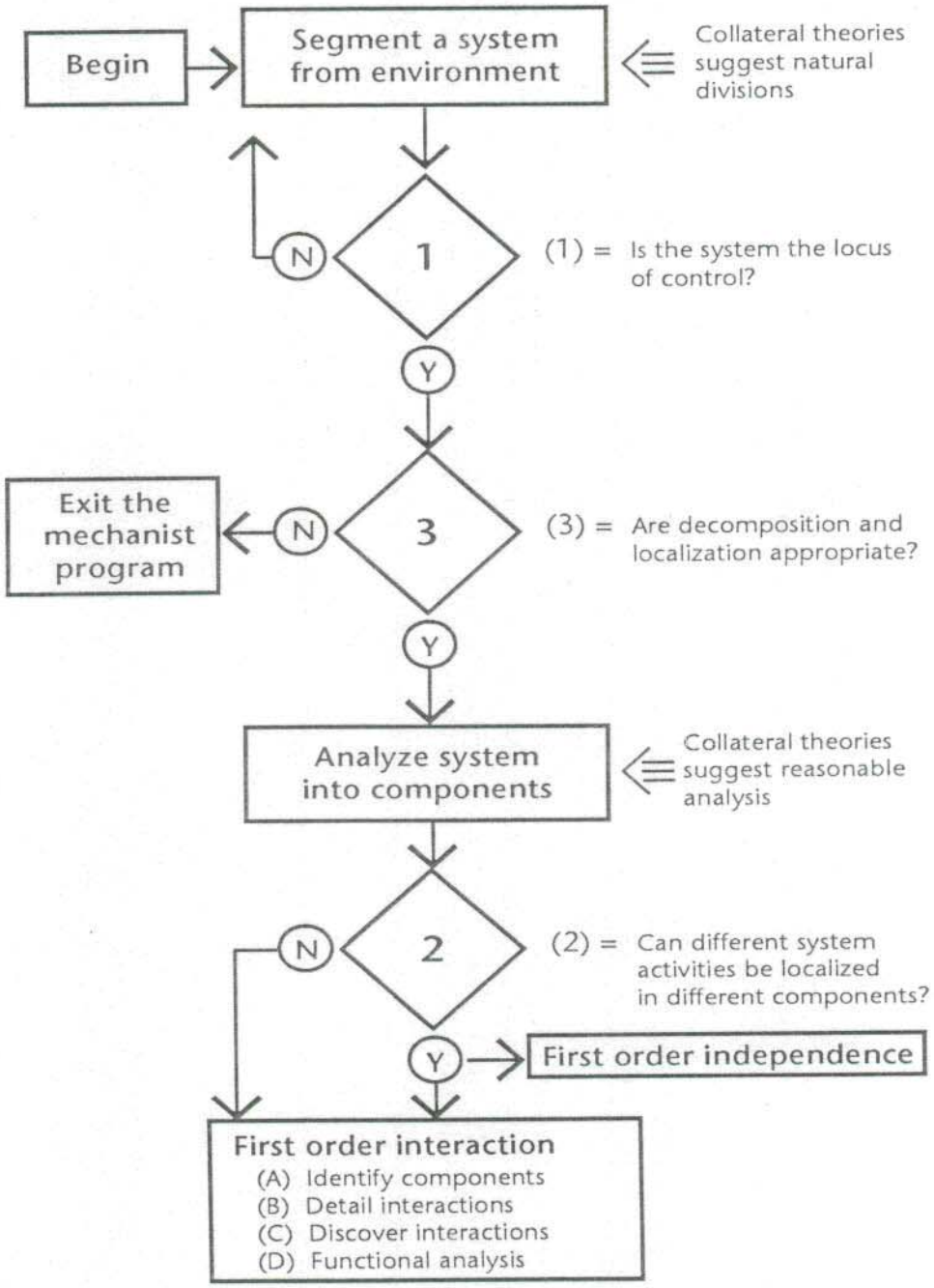


Figure 7.6. A Second Outcome of Decomposition and Localization. Attempts at decomposition and localization can also result in the recognition of a variety of components. In these cases of first order interaction, the organization between components with discrete functions is critical in understanding systemic behavior.