CHAPTER 4

Direct Localization

1. Introduction: Relocating Control

Having "isolated" a system and identified it as a locus of control, the next step is to ask how the system does what it does. The goal is one of identifying and elaborating the mechanisms underlying behavior. A variety of approaches are possible. One that is often employed—especially in the earliest stages of a research program—is the identification of some component of the system that is itself responsible for producing the behavior, still leaving aside the question of how that component produces the effect. This is what we have called direct or simple localization. Responsibility for an effect is localized directly in a single, constituent component of the system. A system with complex capacities must have a complex structure. Direct localization acknowledges this and purports to explain complex capacities as a multiplicity of capacities. That is, direct localization proposes an analysis of the system into a set of components, each responsible for a specialized capacity. With direct localization, interaction among components is assumed to be either insignificant or nonexistent.

The empirical evidence for localization lies in the observed behavior of the system. In some cases, this is no more than correlational evidence; in others, we have experimental intervention in the form of excitatory or inhibitory studies (cf. Chapter 2). However, in each of the cases we will discuss in sections 2 and 3, independent lower-level constraints play a minimal role in the resulting models. In this sense direct localization is localization subject to minimal empirical and theoretical constraints—or, as we will sometimes say, it is *minimally constrained localization*.

Direct localization is prima facie reasonable, if only as a first approximation. Suppose we are confronted with a device that has an unknown structure and a complex output. If we propose to explain how some aspect of its behavior is controlled, one natural question to ask is: What part of the system controls it? We assume that the device is decomposable and that some specific component is responsible for its various behaviors. The practice is common with human institutions, as when we ask what person in a corporation is responsible for a given decision. Likewise, we may ask what part of a computer allows us to store information.

Identifying a component in the system responsible for a particular effect differs from identifying the locus of control *only in the level of analysis*.

Determining the locus of control requires segmenting a system from its environment and ascertaining whether control is internal or external. Direct localization requires segmenting a system into components and isolating some component or components within the system as controlling an effect; it transfers the locus of control for some dimension of system behavior to a lower level of organization and localizes control in a component subsystem. The proximate environment for the subsystem is the rest of the system.

Just as with the identification of a locus of control, direct localization involves a number of important and contentious assumptions. Jointly they are tantamount to assuming the decomposability of the system. Direct localization assumes that there are a number of components in the system, that these components function independently, and that any complexity in the behavior of the system is the effect of isolable subsystems. From the perspective of the behavior of the system, this entails that the various dimensions in its behavior are also relatively independent. Granting these assumptions, direct localization identifies component systems and proposes that one or several parts constitute a lower-level locus of control for each dimension of system behavior. Empirically this requires correlating changes in the behavior of component subsystems with changes in gross behavior, and then showing that changes in the former explain the latter.

In some cases the needed correlations are readily gained without elaborate intervention. This may mean searching out straightforward correlations between traits, as when we find that there is a correlation between smoking and lung cancer, or between performance on IQ tests and scholastic performance.[1] In other cases obtaining the correlations may require moderate intervention, as when we find that there is a correlation between REM sleep and dreaming. Similarly, we may investigate neuroanatomy by magnetic resonance imaging or regional cerebral blood flow in order to ascertain functional properties of the cortex. Finding the correlations may require substantial statistical analysis, as when we use statistical manipulations to separate signal from noise in isolating the activity of single neurons, or when we determine the characteristic signature of regions of the brain in EEG readings. In yet other cases, obtaining the needed correlations requires sophisticated experimental intervention. Finding the correlations may rely on naturally or artificially induced abnormalities, as when we find that there is a correlation between schizophrenia and an excess of dopamine in the central nervous system, or we realize that hemophilia affects only males. In these cases there are a variety of experimental techniques to identify component subsystems that control or modulate system behavior. In one way or another we can alter the behavior of a subsystem and then look for associated changes in the behavior of the system as a whole. As we pointed out in Chapter 2, one

common experimental procedure is to disrupt the performance of the subsystem and then show that there is a corresponding inhibition of the effect one wants to explain. Another alternative is to intensify the activity of the subsystem and then show a corresponding increase of the effect in question. The use of such inhibitory and excitatory techniques is as controversial and difficult as it is common.

Direct localization, if correct, does explain why the system *as a whole* behaves as it does. We shift to a lower level of organization, identifying component parts and organization; then we isolate the component, if there is one, that is responsible for what the system does. If we want to know why an individual is schizophrenic, the presence of elevated dopamine levels is both relevant and important. Direct localization does not, however, provide an ultimate explanation, as it does little more than locate an underlying system within a complex system. Even if direct localization is successful, it tells us only *what* produces the effect, and not *how* it is produced. We may still want to know how the component system produces the effect it does, but that question is deferred rather than answered in seeking a direct localization. More positively, direct localization relocates the problem as one to be answered by research geared to a lower level of organization.

Even though direct localization does not produce a full mechanistic explanation, use of the heuristic is often an important preliminary step in the search for mechanisms. Without isolating a component that exerts control, if there is one, we cannot begin to provide a detailed mechanism. If control turns out to be more complex, involving several components, finding a relevant component may facilitate the search for others. Direct localization is a first pass at parsing the behavior and control of a system into causally significant segments. It is also a common strategy. Whether the resulting model of the system and its organization is right or wrong, this is a strategy that a realistic model of discovery would be ill-advised to neglect. In the sections that follow we will sketch several models incorporating direct localization. In section 2 we turn to phrenology; in section 3 we examine two competing accounts of respiration—those of Heinrich Wieland and Otto Warburg.

2. Phrenology and Cerebral Localization

In the nineteenth century Sir William Hamilton described phrenology as "idiotcy grafted upon empiricism" (cited in Cooter 1984, p. 48). Adam Sedgwick was at least as disparaging, describing it as a "sinkhole of human folly and prating coxcombry" (cited in Young 1970, p. 10). More recent accounts often follow the lead of Hamilton and Sedgwick, treating phrenology as a pseudoscientific enterprise, comparable to the use of water

cures to treat recurring ills, reflecting nineteenth-century social fads and fashion, but hardly deserving treatment as a serious scientific view. This view has no credibility in light of historical treatments of phrenology by, among others, John D. Davies (1955), Robert M. Young (1970), Barry Barnes (1974), David de Guistino (1975), Stephen Shapin (1975, 1979), and Roger Cooter (1980, 1984). Though in the later phases of the movement phrenology was marked by a tendency toward popularization, it is a caricature to treat it as merely a piece of "Continental quackery," as it was often described. Moreover, it is important to recognize that the dichotomy between science and pseudoscience is often little more than a rhetorical flourish designed to enforce a particular point of view (cf. Mendelsohn 1977; for a contrary view, see Thagard 1988, ch. 10)—an ideologically conservative view in particular (cf. Cooter 1980). The actual opposition to phrenology was largely grounded in the antimaterialist and antispeculative movements of the early nineteenth century. In France, under the leadership of Georges Cuvier and the explicit assaults by Flourens and Magendie, phrenology hardly had a hospitable climate. In Britain it fared little better. Indeed, the phrenological societies that flourished in Britain during the early decades of the nineteenth century can be seen as a response to the exclusion of phrenological research from the Royal Societies and the British Association for the Advancement of Science, which were under the conservative leadership of William Whewell and Adam Sedgwick.[2] Despite the parody of phrenology promoted by its opponents, Franz Joseph Gall (1758–1828) and Johan Gaspar Spurzheim's (1776–1832) emphasis was clearly empirical, even though it was based on comparative anatomy rather than experimental ablations or the study of neurological deficits.[3]

In understanding phrenology and its pretensions it is useful to understand its historical context, and particularly its debt to one strain of Cartesianism. Cartesian physiology established that the nervous system was to be understood in mechanistic terms: its functioning is mechanical and automatic, requiring the intervention of no conscious agent. Cartesianism also bequeathed to us the doctrine that the brain is "the organ of the mind": all action and perception is mediated by the brain, and it is only through this organ that the mind influences the body.[4] According to this view the soul no longer animated the entire body, but rather exerted its influence through the brain. The overall effect of the Cartesian orthodoxy was no doubt salutary for physiology, as it freed physiological investigation from the vitalistic assumptions of its predecessors; however, the brain, though mechanical in operation, was regarded as a single functional unit, largely or wholly undifferentiated as to function. The mind was taken to animate the brain as it once had animated the body. At the same time, Cartesian metaphysics exempted the actions of the soul from the domain

of empirical science; the Cartesian mind was nonmechanical in its operation. The investigation of *cognitive* activity—of the "higher functions"—became a matter mainly for introspection and speculation.

The assault on the citadel of Cartesianism did not begin in earnest until the nineteenth century. Gall led the break with the tradition by maintaining that the brain is *not* a homogeneous unit, but consists of a variety of organs, or centers, each subserving specific intellectual and moral (that is, practical) functions. These organs were localized in the cerebral hemispheres and thus were distinct from those organs subserving both the vital functions and the affections, which he localized in "lower" portions of the brain. The various intellectual and moral functions were, in turn, diverse from one another and were likewise localizable in discrete portions of the cerebral hemispheres. Gall's more infamous commitment to craniology was, of course, coupled with this view of the brain as a network of relatively independent organs. He held that the skull was a rather malleable structure which responded to the growth of the underlying nervous tissue and, accordingly, could be used as an index of the size of the underlying organs. Since he believed that the size of the underlying organs determined the extent of the respective intellectual abilities, the shape of the skull could be used as an empirical measure of the intellectual abilities (see Figure 4.1). Craniology was the source of phrenology's popularity among Gall's contemporaries, as it is the source of its derision among ours. Yet Gall's enduring contribution was his organology. It was also the focus of the main controversies surrounding phrenology among his contemporaries, as we will see in Chapter 5.

Phrenology was carried to England by Gall's collaborator, and the person who also coined the term *phrenology*, Spurzheim. Spurzheim made minor modifications to the organology (much to Gall's dismay), and it eventually gained serious attention in both the United States and Great Britain.[5] At the height of its popularity in the 1830s, the phrenological movement encompassed between forty and fifty independent phrenological societies in the United States alone. Under the leadership of George Combe in Britain and Orson Squire Fowler in the United States, it left its mark not only on medicine but on education and politics as well.[6]

In any of its incarnations and variations there is a tripartite commitment at the heart of phrenology: the cerebral hemispheres are held to be a collection of relatively independent organs which subserve the primary intellectual functions; the size of these respective organs is correlated with, and the basis for, differences in abilities; and, finally, the size of these organs, and thus the differences in abilities they reflect, can be measured by examining the shape of the skull. The capacities of individuals are explained as the products of organs localized in the cerebral lobes,

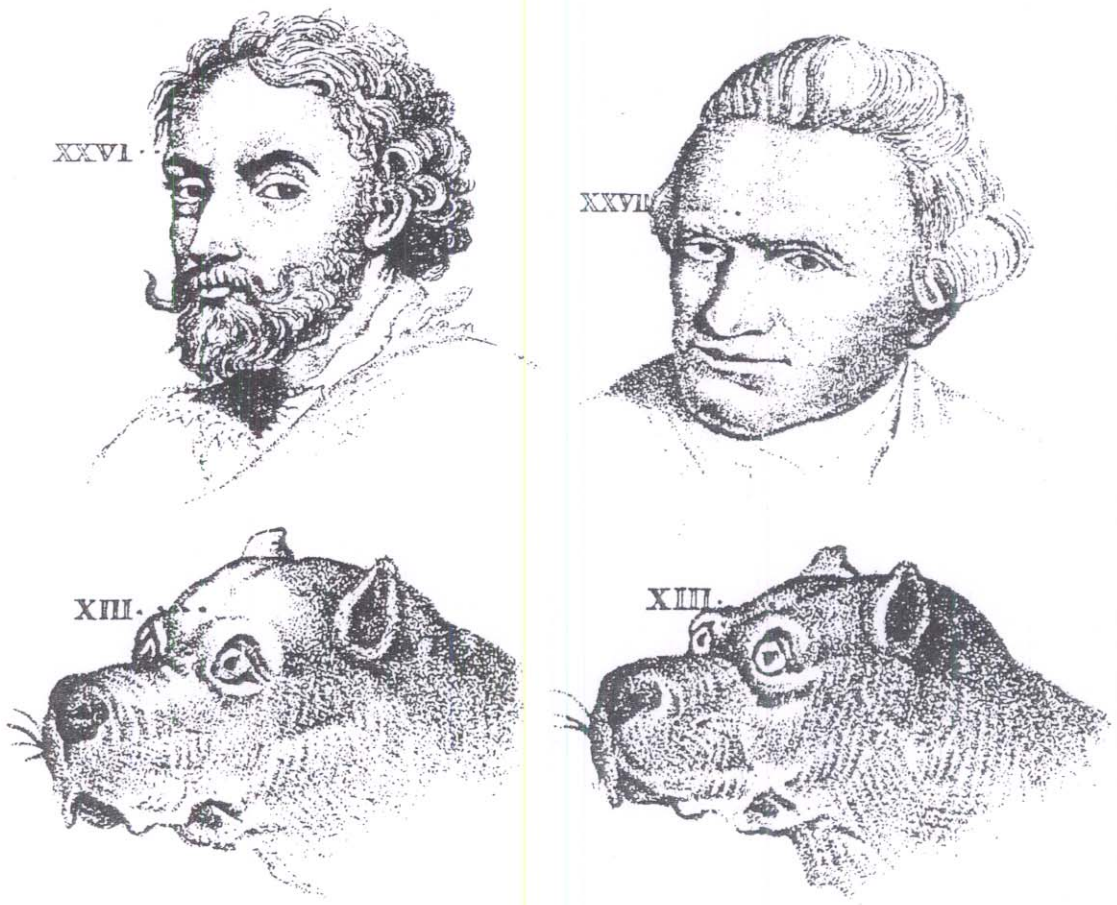

Figure 4.1. Phrenological Faculties and Cranial Localization. These craniological representations are reproduced from an American reprinting of Spurzheim's *Phrenology, or the Doctrine of the Mental Phenomena* (1833). It is important that phrenological analyses incorporate both interspecific and intraspecific comparisons. What are called *affections* tend to be posterior, including, for example, propensities toward combativeness or secretiveness, as well as sentiments such as self-esteem and benevolence. The intellectual powers are anterior, including such things as senses of time or language.

though the activities of these organs were not themselves subjected to examination or explanation. Together these assumptions embodied a research program intent on transforming psychology into a more "biological" study and one that was less speculative than its competitors. This was true of the craniology no less than the organology. As Young explains, Gall "was wrong, but his [cranioscopic] hypothesis was extremely plausible at the beginning of the last century, and it played a very important part in the transition from speculations about unverifiable physiological homogeneity to the experimental study of the brain" (1970, pp. 14–15). Let us look briefly at each of the three phrenological commitments and their contributions to the program of phrenology.

First, it was emphasized that the exercise of psychological faculties depended on their physiological realization: "The faculties and propensities of man have their seat in the brain" (Gall 1810–1819, 1:10). Spurzheim later described this as the "First Principle of Phrenology": "The brain is the organ of the mind" (1832, p. 6). In support of the principle, Spurzheim marshaled a variety of what were by then commonplace observations: the brain is necessary for feeling and thought; defective manifestations of thought accompany defective organization; and healthy development of the brain suffices for "energetic affective and intellectual powers" (ibid.). Together these were taken to imply the dependence of mental functioning on brain development. Phrenologists differed among themselves over the kind of organization to be found in the brain and the relation between mental faculties. Gall tended, for example, to deny any interaction whatsoever between faculties, while Spurzheim thought interaction was an important feature in explaining behavior.[7] None, however, challenged phrenology's "First Principle."

Second, and most centrally, phrenologists maintained that there was a correlation between the activities of the several faculties and the size of the appropriate physiological organs. This was Spurzheim's "Second Principle of Phrenology": "The mind manifests a plurality of faculties, each individually by means of a particular organic apparatus" (1832, p. 10). O. S. Fowler, the most prominent of the American phrenologists, ties the first two principles together:

> Phrenology points out those connexions and relations which exist between *the conditions and developments of the brain, and the manifestations of the mind*, discovering each from an observation of the other. Its one distinctive characteristic feature is, that each class of mental functions is manifested by means of a given portion of the brain, called an organ, the size of which is the measure of the power of the function. (1848, p. 5)

There was considerable variation concerning exactly how many and what these faculties were (for one taxonomy of the faculties, derived from Spurzheim, see Table 4.1), but it was agreed that the *faculties* were the species of affection or intellection and that the *organs* were the means by which the faculties of the mind were made manifest. A specialized apparatus for each function seemed to Gall and his followers to be a natural corollary of an analogy between the brain and organic structure. As George Combe, the leader of the Edinburgh phrenologists, said,

> Any theory, founded upon the notion of a single organ, is uniformly at variance with all that is ascertained to be fact in the philosophy of mind; . . . on the other

1. Affective Faculties
- 1.1 "Propensities," the desires due to instinct
  - 1.1.1 Vivativeness, or the instinct for survival
  - 1.1.2 Alimentiveness, or the appetite for food
  - 1.1.3 Destructiveness
  - 1.1.4 Amativeness, or physical love
  - 1.1.5 Philoprogenitiveness, or caring for offspring
  - 1.1.6 Adhesiveness, or bonding (friendship)
  - 1.1.7 Inhabitiveness, or the tendency to be attracted to specific types of localities
  - 1.1.8 Combativeness, or the tendency to fight
  - 1.1.9 Secretiveness
  - 1.1.10 Acquisitiveness
  - 1.1.11 Constructiveness
- 1.2 "Sentiments," which are not confined to inclination
  - 1.2.1 Cautiousness
  - 1.2.2 Love of Approbation
  - 1.2.3 Self-Esteem
  - 1.2.4 Benevolence
  - 1.2.5 Reverence
  - 1.2.6 Firmness
  - 1.2.7 Conscientiousness
  - 1.2.8 Hope
  - 1.2.9 Marvelousness
  - 1.2.10 Ideality, or enthusiasm
  - 1.2.11 Mirthfulness
  - 1.2.12 Imitation

2. Intellectual Faculties
- 2.1 External Senses
  - 2.1.1 Feeling, as in pain, pleasure, temperature, etc.
  - 2.1.2 Taste
  - 2.1.3 Smell
  - 2.1.4 Hearing
  - 2.1.5 Sight
- 2.2 Perceptive Faculties
  - 2.2.1 Individuality, producing the idea of being or existence
  - 2.2.2 Configuration, producing knowledge of patterns
  - 2.2.3 Size
  - 2.2.4 Weight
  - 2.2.5 Coloring
  - 2.2.6 Locality, producing knowledge of "the relative localities of external objects"
  - 2.2.7 Order
  - 2.2.8 Calculation
  - 2.2.9 Eventuality
  - 2.2.10 Time
  - 2.2.11 Tune
  - 2.2.12 Language, or the "power of knowing artificial signs"
- 2.3 "Reflective" faculties, which operate on other sensations and notions; constitutive of Reason.
  - 2.3.1 Comparison
  - 2.3.2 Causality

Table 4.1. The Varieties of Mental Powers According to Spurzheim.

hand, the principle of a plurality of organs, while it satisfactorily explains *most* of these facts, is consistent with *all* of them. (1835, p. 19)

Discrete functions and abilities require distinct organs. This isomorphism, however, was explicitly restricted by phrenologists to "fundamental" or "primitive" faculties—those that maintain substantial independence from one another. In Gall's view the flaw in speculative faculty psychology was the failure to focus on these faculties. This was, according to Gall, also the source of the failure to recognize cerebral localization. As Spurzheim said, "The essence . . . of every faculty is always perceptible, . . . the essential nature of each primary power is invariable, and no organ can produce two species of tendencies" (1832, p. 21). The empirical implication is that there are a number of faculties between which there is relatively little correlation. For example, the sense of taste may be highly developed though the sense of hearing is not; similarly, the tendency toward destructiveness may be lacking while that toward combativeness is not.

Third and finally, the skull reflected the form of the brain. Spurzheim described it this way: "From birth and through mature years, up to the period when the faculties fall into decay, the size and form of the brain and its parts may be determined by the size and form of the external head" (ibid., p. 15). In Gall's view, any "difference in the form of heads is occasioned by the difference in the form of the brains" (1810–1819, 1:55). This final phrenological commitment provided the empirical criterion for localization. Phrenologists sought correlations between cranial structure and behavioral propensities, thinking that the only reasonable explanation for the correlations would be based on increased development of the appropriate brain structures. This study included comparisons of individuals: those having some pronounced or unusual capacity were examined for peculiar or extraordinary prominences, and those lacking capacities were inspected for the lack of corresponding cranial structures.[8] The craniological assumption was important to phrenology as an operational criterion. Because cranial structure was supposed to reflect brain structure, any correlations between cranial structure and special abilities was taken to be significant.

Organology was central to phrenological thought; it constituted an assumption of discrete faculties and their localization in correspondingly discrete regions of the cortex. As Herbert Spencer wrote, "A FUNCTION to each organ, and each organ to its own function, is the law of all organization" (1851, p. 274). In the simplest of cases, exemplified in Gall's writings, it was assumed that the organization was aggregative. Gall assumed that there was no significant interaction between faculties and, accordingly, that complex abilities were simply aggregates of simple abilities. Spurzheim's most significant departure from his teacher's views was pre-

cisely in the form of organization he allowed. On the one hand Spurzheim emphasized that many effects are due to "the mutual influence of the primitive faculties" (1832, p. 13). In his hands the organization was no longer aggregative, and though the primitive faculties maintained discrete localization in the brain, they were no longer functionally distinct.[9] The relative simplicity of either form of organization guaranteed that, as we shift to a lower level of organization in looking at the brain, we could transfer control for any basic cognitive capacity to a discrete unit at the lower level. Gall, on the other hand, supported the view that we have wholly independent organs with independent capacities. This was direct localization in its simplest and starkest form.

3. Competing Models of Cellular Respiration

We now turn to two theories involving attempts to localize cellular respiration, or biological oxidation, in different biochemical components of the cell. By focusing on an ongoing dispute between two theories within a research tradition that presupposed direct localization, we can see how such controversies depend upon common assumptions. This dispute differs from the previous one in crucial ways. Unlike the phrenologists, researchers in biochemistry did not rely simply on an observed correlation; they also developed experimental techniques to evaluate the correctness of their claims to localization.

Pflüger's research established that respiration occurred within the cell. This was not in itself an attempt to explain how respiration was accomplished. An explanation was needed, though, since the substances involved—carbon, hydrogen, and oxygen—do not readily react at the temperatures found in the cell. Intracellular respiration therefore appeared anomalous. What was required was an account of how the cell was able to facilitate respiration; that is, the problem was how to explain the reactions involved in oxidation given the intracellular temperatures. Researchers assumed that there was a simple mechanism, a catalyst, that facilitated oxidation. The problem then, became one of identifying and localizing this catalyst. Investigations into processes such as fermentation had led to the introduction of the term *enzyme* for a catalyst found within a cell.[10] Because the enzymes were identified in terms of their specific effects, the central differences between competing models were to be found in what activities or functions the accounts proposed for these enzymes.

Attempts to explain biological oxidation in chemical terms began in the nineteenth century using two very different approaches. One, by Schoenbein and Traube, focused on an alteration of molecular oxygen to make it more likely to react with the substrate. Schoenbein's proposals stemmed

from his discovery of ozone. In 1845 he showed that ozone could oxidize guaiacum, and in 1848 he showed that potato roots had the same effect. From these results he constructed a general theory in which the formation of ozone, which he took to be an especially active form of oxygen, was an intermediate step in biological oxidation. Traube also developed an account that focused on oxygen, but instead of proposing a specialized form of oxygen he proposed a reaction in which ordinary oxygen was transported to a substrate. In Traube's models this typically involved the formation of hydrogen peroxide from water and molecular oxygen, which in turn would oxidize the substrate. In contrast to both of these models, Hoppe-Seyler developed a model according to which the crucial step was the formation of molecular hydrogen, which in turn possessed unusual capacities to react with oxygen (for more detail, see Kastle 1910).

These nineteenth-century proposals constitute direct localizations in that they propose a single mechanism for explaining biological oxidation. Each of the models postulated single enzymes with specific functions, based on an understanding of cellular function. However, because of limitations on experimental techniques, it was difficult to elaborate on these models. Each, moreover, faced serious empirical and theoretical problems. We turn now, though, to two analogous models advanced and developed in active research programs in the early twentieth century. These differ from each other fundamentally on their understanding of the action of the enzymes in catalyzing the oxidation reactions of cellular respiration. Wieland, later supported by Thunberg, argued for a process in which enzymes removed hydrogen from substrates. The key to the proposal—one reminiscent of Hoppe-Seyler—is the enzyme that removes the hydrogen from the substrate, thereby allowing it to react with oxygen. The alternative model, by Warburg, incorporates *oxygen activation*, according to which oxygen, once activated on the surface of his proposed agent, the *Atmungsferment*, would react directly with the substrate.

These alternative models share a simple structure. Each posits a single enzyme with a specific effect. Since each enzyme could, in principle, account for the net effect of respiration, experimental techniques were introduced to determine the detailed conditions under which respiration occurred. There was heated controversy surrounding this issue until the mid-1920s. We now know that both Wieland and Warburg identified an important functional component that figures in cellular oxidation. They both, however, attributed responsibility for the total oxidation to a *single* component, when in reality *each* component figures as a constituent function in the overall process; and neither researcher was willing to consider a more complex organization. It was not until the 1920s that investigators began to appreciate how the two accounts could be viewed as identifying components of a much more complex process; and it was only in the 1930s

that the basic architecture of this more complex process was identified. Our focus in the remainder of this section will be on the period prior to reconciliation and on the arguments that were used to support the competing models of Wieland and Warburg.

Wieland's Localization of Respiration in Dehydrogenases

Wieland's initial research focus was not on biological oxidation, but on oxidations performed by inorganic catalysts like palladium black, a reaction first studied in the early nineteenth century by Humphry Davy. Wieland studied this catalysis, showing that when no oxygen was present, palladium could remove hydrogen from many compounds. This reaction, however, could only proceed for a short time—until the palladium was saturated with hydrogen. This suggested that oxygen was required *only* to receive the hydrogen released from the substrate by the palladium. It was important, but only secondarily so. To demonstrate this Wieland employed methylene blue (a readily reduced dye), showing that when it was available to receive the hydrogen from the palladium the reaction could continue even in the absence of oxygen. Wieland referred to both the oxygen and the methylene blue as simply "hydrogen acceptors," indicating that they played a basically passive role in the reaction. He thus contended that oxidation consisted, in such cases, not in the addition of oxygen to the substrate, but in the removal of hydrogen:

> By this method of considering the matter, the catalytic action of the platinum or palladium in these processes does not occur by the metal activating a molecule of oxygen (with intermediate formation of a peroxide), but much more probably by the metal activating hydrogen, as is believed to occur in the purification of detonating gas. (1913, p. 3327)

Thus, Wieland proposed the following general scheme for oxidation of a substrate (R) by palladium (Pd), with methylene blue (Mb) serving as the hydrogen acceptor:

$$RH_2 + Pd \Rightarrow R + PdH_2$$
$$PdH_2 + Mb \Rightarrow Pd + MbH_2$$

A number of other oxidation reactions, however, seemed more plausibly construed as involving the *addition* of an oxygen atom to the substrate. For example, the overall reaction in the case of oxidizing an aldehyde can be represented as

$$RCHO \Rightarrow RCOOH.$$

Aldehydes do not possess two hydrogen atoms to be removed. Wieland argued, however, that these reactions still followed the same scheme and

required an intermediate process of hydration. He thus proposed the following for the oxidation of an aldehyde:

$$RCHO + H_2O \Rightarrow RCH(OH)_2 \Rightarrow RCOOH + H_2$$

Wieland similarly suggested that the oxidation of carbon monoxide involved an initial hydration to form formic acid (CHOOH), followed by dehydrogenation to leave carbon dioxide. He maintained that *all* oxidations consisted simply in the removal of hydrogen and did not necessarily involve oxygen.

The contention that oxidations were processes of dehydrogenation prepared Wieland to draw a connection between oxidation and reduction. These processes had previously been conceived as independent, with oxidation involving the uptake of oxygen. In Wieland's scheme, though, reduction and oxidation became "two expressions of one process of dehydrogenation" (ibid., p. 3340). The hydrogen acceptor, whether oxygen or methylene blue, was reduced by the hydrogen removed from the substrate being oxidized:

> If we consider oxidation processes as dehydrogenations, as the foregoing results have indicated exactly, at least for some important cases, then we have a reduction process at the same time, since the hydrogen activated by the ferment must be taken up by some sort of acceptor. (Ibid., p. 3339)

It would follow that no substance can be oxidized without another being reduced. According to Wieland's model, the oxygen that is added to the substrate in oxidation, or contained in the carbon dioxide or water released, does not come from molecular oxygen, but rather from either the original substrate or water, and is of only secondary importance.

Wieland then took up biological oxidations to show that they could be accounted for in a similar way. This extension of models of reduction to oxidation makes Wieland's work a particularly interesting case of direct localization. Reduction was thought to be a chemical process. By treating oxidation as an analogue of reduction, Wieland sought a chemical explanation for what was then thought of as a biological process. Wieland's strategy was to posit an agent in the cell that played the same role as palladium black in the purely chemical oxidations he had studied. He intended to show that biological substrates, such as grape juice, could also be dehydrogenated—that is, oxidized—with palladium black, even in the absence of oxygen, provided another substance, such as methylene blue, subsequently removed the hydrogen from the palladium. Moreover, he showed that the reaction began, even without methylene blue, "with a rich formation of carbon dioxide." The reaction predictably slowed as the metal became saturated, and resumed when methylene blue was supplied.

Recognizing that "this is still only an imitation of the biological oxidation process when it is obtained with a substance foreign to the cell as the catalyst," Wieland demonstrated that organic ferments could also function with hydrogen acceptors other than oxygen—again, most commonly, methylene blue. He thus showed that bacteria could oxidize ethanol and acetaldehyde to acetic acid if oxygen was lacking but methylene blue was provided. The fact that this reaction could occur without oxygen convinced Wieland that biological oxidations involved the same process—the removal of hydrogen from the substrate—as did chemical oxidations with palladium. Oxygen was inessential. Wieland restated his basic argument in *On the Mechanism of Oxidation*:

> As it can be proved that one and the same enzyme system uses in the one case molecular oxygen, and in the next a quinone derivative to fix the hydrogen which has been removed, it follows that the reaction mechanism must be similar in both cases. Common to both is hydrogenation of an unsaturated molecule, namely oxygen or quinone. This uniformity in mode of action cannot be attributed to an activation of the oxygen, but is satisfactorily explained by the assumption that the hydrogen in the substrate is activated. (1932, p. 30)

Wieland was not able to identify directly the catalytic agent he claimed was involved in oxidation within living cells. These agents could only be identified functionally in terms of the reactions they catalyzed. Since he could not isolate and study the enzymes, he had to proceed indirectly and examine the overall reactions occurring in the cells under a variety of circumstances. The circumstances that proved critical were those that showed oxygen was not necessarily involved in respiration and that water was required for some oxidations. In addition to studies of this form, Wieland tried to show the similarities between his model system involving palladium black and the biological cases. Thus, he attempted to determine the kinetics of milk dehydrogenases and study the role of hydrogen peroxide in these reactions in order to compare them to his model systems (ibid., ch. 4). The parallels between the behavior of biological systems and the predictions he made on the basis of his dehydrogenation model were, he thought, convincing evidence that hydrogen rather than oxygen was activated in these reactions.

Except for one critical response by Bach (1913), Wieland's proposal did not initially attract much attention, negative or positive. One factor that helps to explain this is Wieland's role as an outlier to the emerging biochemical community. He was not principally concerned with reactions in living systems, but more generally with organic chemistry.[11] Accordingly, Wieland's approach to oxidation was more typical of an organic chemist; his focus was principally on the structural changes that occur in oxidation.

Moreover, he was trying to oxidize organic compounds using palladium as a catalyst. When he attempted to extend his theory to biological oxidations, he concentrated on tracing the kinetics of the biological reactions and showing that they corresponded to the kinetics of reactions achieved in his model system. As a result he did not construe the problem in the same way as had more biologically oriented researchers, who focused on the enzymes potentially involved and tried to identify them either through purification or inhibition.[12]

There was already recorded evidence in physiological chemistry that could be interpreted to support the idea that oxidation occurs through dehydrogenation. Wieland, however, did *not* establish the auxiliary connections; they were developed a few years later by Torsten Thunberg, who saw the relevance of Wieland's scheme to the biological research he was conducting. Battelli and Stern (1911) had worked with the oxidation of succinic acid. In accordance with the view that oxidation involves a direct reaction of a substrate with oxygen, they took the product of this oxidation to be malic acid. Einbeck (1914) showed, though, that the first step in oxidation was the formation of fumaric acid. The reactions can be represented as follows:

$$\underset{\text{[succinic acid]}}{HOOC-CH_2-CH_2-COOH} \Rightarrow \underset{\text{[fumaric acid]}}{HOOC-CH=CH-COOH} \Rightarrow \underset{\text{[malic acid]}}{HOOC-CH_2-CH(OH)-COOH}$$

The intermediate step is curious from the perspective of traditional theories of oxidation, but it makes perfect sense from the perspective of Wieland's dehydrogenation theory: fumaric acid is dehydrogenated succinic acid; malic acid is formed by hydration of fumaric acid.

To defend his account, Thunberg required not just an interpretation of data in accord with Wieland's theory, but experimental evidence showing that the biological reactions did indeed go through the kind of hydration and dehydrogenation Wieland's theory required. Biochemists were already in the process of developing experimental tools for analyzing such stepwise reactions. Knoop (1904) and Dakin (1912) had proposed a scheme for fatty acid metabolism that involved a sequence of oxidations of the beta carbon.[13] Demonstrating this scheme depended on isolating the proposed intermediary substances. Since these substances tended to be metabolized as rapidly as they were formed, however, this proved difficult. Thus, techniques had to be developed to trap intermediaries or interrupt the process at certain stages to identify the intermediate products that were produced.

Thunberg's investigations stemmed from his observation that succinic acid—the final product formed in the oxidation of fatty acids with active oxidizing agents such as nitric acid—could be readily oxidized by living tissues. He constructed a device (later known as the Thunberg tube) which was evacuated of air. Into it he put muscle substance and methylene blue. When succinic acid was added to the tube, the methylene blue was rapidly decolorized. He concluded that the enzymes of the muscle tissue had activated the hydrogen in succinic acid (Thunberg 1916). He then needed to demonstrate the stepwise character of this oxidation. In his earlier experiments, Thunberg had found that addition of other substances could also increase oxygen uptake of tissues. He now tested numerous substances in the Thunberg tube and showed that malic acid, fumaric acid, citric acid, and lactic acid, among others, could all be dehydrogenated with methylene blue. Although Thunberg could not yet isolate the enzymes responsible for these effects, he *was* able to show that the capacity of the cell to carry out various of these reactions was differentially affected by heat. Knowing that different degrees of heat could destroy or inhibit catalytic capacities of enzymes, he argued that there were discrete enzymes responsible for the different reactions (1920; for a review, see Thunberg 1930).

Thunberg's ability to incapacitate selective enzymes responsible for different steps added critical support to Wieland's claim that oxidation was carried out by specific localized agents in the cell. In particular, Thunberg saw this as evidence against the view that the cell structure as a whole explained the ability of cells to carry out these reactions—a view he saw as a vestige of nineteenth-century protoplasm theory. Thunberg allowed that cell structure could effect the harmonious linking of reactions and might play a role in "the transformation of chemical energy into other forms of energy." He contended, though, that the ability to carry out oxidation with destroyed cells counted against the need for such structure for the basic reactions:

> The dilatory conditions to which muscle mass has been subjected speak against the supposed importance of the cell structure to the reactions in question. The mincing of the muscle cells and the treatment with distilled water are factors which must derange the polyphasic system of the muscle cells, i.e., their structure. (1930, p. 326)

He maintained that a highly localized agent, one that was not disturbed by destroying cell structure but which was destroyed by heat, was the true agent of such oxidations. Thunberg's argument paralleled Wieland's in the claim that oxygen was not needed for oxidation. Both cases relied on showing that the reaction continued even when the supposedly critical

factor (cell structure or oxygen, as the case may be) was removed. The central factor must therefore be something else, or so they argued.

In positing the explanation of oxidation by hydrogen activation, the Wieland-Thunberg scheme is a straightforward case of direct localization. Thunberg's account is more complex than Wieland's in proposing a chain of such oxidations in the cell, each due to a separate enzyme. Every step in oxidation is attributed to a single, specific agent, which executes its function in the cell by removing two hydrogen molecules from the substrate and then uniting the hydrogen with available acceptors. Thus, a direct localization is offered for each oxidation in the cell. In Thunberg's scheme, complex reactions are not explained in terms of an integrated system of reactions, but are decomposed into a series of simple and self-contained steps, which are linearly ordered and mediated by single, specific agents.

Neither Wieland nor Thunberg offered any independent evidence regarding the nature of the enzymes mediating hydrogen activation. Both submitted models based on the behavior of cells or cell extracts; neither suggested chemical mechanisms to explain how the proposed enzymes catalyzed cellular respiration. As we have said, an explanation of this sort is incomplete even when it is correct. Without some account of how the enzymes are capable of performing the dehydrogenations attributed to them, the scheme is not yet fully mechanistic. In the 1920s Wieland did finally propose such an account:

> It may be considered that partial valencies are concerned in the union of adsorbing and adsorbed substances and that the utilization of these partial valencies causes a readjustment of the valency relationships in the molecule of the adsorbent. An unmistakable chemical reaction occurs during the adsorption of hydrogen on platinum or palladium, and it is accompanied by a very considerable evolution of heat. Here we see the effect of the saturation of the partial valencies of the hydrogen molecule. The adsorption compound might be expressed as follows, where the number of palladium molecules involved is not expressed.
>
> $H\ Pd_1 \ldots Pd_n\ H$
>
> By the saturation of the partial valencies, the link between the two [terminal] hydrogen atoms is weakened, and in this we see the reason for the activation of the hydrogen. During hydrogenation, this complex adsorption compound reacts with the similarly adsorbed hydrogen acceptor whereby the active hydrogen gives up its valency linkage and performs the task prescribed for it by the thermodynamic conditions. The continuous removal of the product of hydrogenation from the catalyst by fresh hydrogen produces the endless repetition of the surface reaction and, accordingly, the catalytic effect. (1922, p. 515)

For hydrogen bound within molecules, Wieland proposed that whole molecules attached to palladium. These palladium-enriched compounds involved a reduced internal valency in the molecule, allowing the hydrogen to join directly with the palladium, and from there on with other acceptors. Wieland's suggestion was no more than a speculation concerning how catalysts *might* perform their functions. Developing an empirically motivated model was a task for the future.

Warburg's Localization of Respiration in Membrane Iron

An alternative, but equally localizationist, model for respiration and oxidation was offered by Otto Warburg in the period 1910–1915. Like many of his nineteenth-century predecessors, Warburg construed oxygen as the active agent in oxidation. In the earliest part of this period, he emphasized structure and developed a view that accorded with the then-emerging colloidal conception of respiration, which was markedly opposed to the enzyme-based models. He proposed that the oxygen was activated by a structure on the membrane surfaces within the cell. He called this surface agent the Atmungsferment, viewing it as a general catalyst for oxidation and not as more specifically involved in certain reactions.

Warburg's early departure from the main tradition of enzyme accounts, emphasizing organization rather than component analysis, is explained partly by his background and his principal objectives as a biochemist. Warburg's work focused on cancer. The continuous division of cancer cells led Warburg to examine the process of cell division. His closest affiliations were therefore with researchers in experimental cytology and embryology, such as Loeb and Verworn, rather than with the emerging orthodoxy in biochemistry, which focused primarily on the extracts that could be studied in isolation from living cells (Kohler 1973a). Some of Warburg's earliest experimental work was on sea urchin eggs, in which he investigated Loeb's claim that the nucleus was the locus of metabolic activity. He concluded, contrary to Loeb, that membrane surfaces rather than the cell nucleus were the locus of respiration. He established this by showing that alkaline solutions increased the rate of respiration. These solutions only affect the acidity of the membrane, not the acidity of the protoplasm. It followed, he thought, that membranes played the key role in respiration. In a contrary fashion, fatty acids and organic solvents decreased the rate of respiration. Warburg interpreted this as resulting from their effect on the membrane. By a combined use of excitatory and inhibitory methods, Warburg claimed to establish that membranes are the components responsible for respiration in the cell.

Warburg's model clearly incorporated a direct localization, but in appealing to a membrane surface rather than an enzyme, it was quite differ-

ent from Wieland's enzyme model: it fixed a different locus of control. It also attracted a distinct group of adherents in the early 1920s. Kohler, for example, explained the attractiveness of Warburg's theory as due to growing disillusionment with the enzyme view:

> Despite a steady stream of new discoveries, biochemists grew less certain that the basic mechanisms of life were simple enzyme reactions. The persistent failure to isolate a pure enzyme raised doubts that enzymes were proteins or that proteins were definite chemical substances. The blossoming popularity of colloidal chemistry at the time provided an attractive alternative view. . . . By the 1920s enzymes were widely regarded as small organic molecules or metal ions adsorbed on an ill-defined mixture of colloidal 'carrier' proteins. This was the prevailing mood and beliefs of the period in which Warburg's *Atmungsferment* flourished, and Warburg's view was very much in harmony with it. (1973a, p. 174)

One reason for this growing dissatisfaction with enzyme models was that although cell extracts in isolation could perform many crucial biological reactions, they performed them more slowly than did whole living cells. The natural conclusion, as Warburg saw, was that the reduced respiration in extracts from liver cells was due to the destruction of the intact structure of living cells. It is *not* the presence of chemical constituents that explains biological processes, but cellular structure:

> The biologists who work with press-juices of organs have generally observed that the important cellular functions are no longer present in cell extracts. It was generally presumed that structure was a necessary condition for most biological reactions. Knowing now the importance of cell surfaces for expiation, such results are no longer surprising. (1910, p. 328)

As we will explain in Chapter 7, similar retardation of the rate of reaction was found when fermentation was carried out in cell-free extracts. Warburg, in fact, challenged the enzyme theory of fermentation, arguing that it neglected the role of cell structure. He argued that while the "ferments," or enzymes, did figure in such reactions, it was the adsorption of the ferment onto the cell membrane that played the crucial role in accelerating the reaction (1912).

While Warburg argued against localization of the causal factors responsible for physiological functions *in enzymes*, he nonetheless pursued a localizationist strategy—and, indeed, a *direct* localizationist strategy. This showed up in his experimental approach, which was directed to determining whether something was or was not *the agent* responsible for a process. Thus, he studied the effects of a number of narcotics such as ethyl urethane to determine how they interrupted cellular respiration. He showed that the more lipid-soluble the narcotics, the greater their inhibitory ef-

fect. He concluded that it was not the lipid phase but the solid particles that were responsible for the increased effectiveness of these narcotics.[14] Instead of treating the poisoning as due to an alteration of the overall physical state of the membrane, he now attributed it to the adsorption of the narcotic on the solid surfaces, where it blocked access to a physical-chemical unit.

Otto Meyerhof, then working in Warburg's laboratory, made an important discovery at this point: citric acid and tartaric acid halted respiration in sea urchins. These acids were known to find and remove iron molecules. That suggested to Warburg that iron embedded in the cell membrane might be the crucial factor in explaining respiration. The fact that adding iron salts increased sea urchin respiration further supported this suggestion. As a consequence, Warburg proposed "that the oxygen respiration in the egg is an iron catalysis; that the oxygen consumed in the respiratory process is taken up initially by dissolved or adsorbed ferrous iron" (1914a, pp. 253–54). He now proposed that Atmungsferment consisted of ferrous iron, adsorbed onto the inner surfaces of cell membranes. Once oxidized, the iron would convert to what Warburg termed the "ferric state" and would serve to oxidize molecules of substrate, which were also adsorbed onto the cell surfaces. Even as the specific character of his explanations changed, Warburg's strategy remained localizationist, despite his opposition to enzymatic views.

Experimentally, Warburg emphasized the interruption of oxidation and attributed the overall reaction to *whatever* was thereby affected. Having concluded, on the basis of experimental disruption, that ferrous iron was the agent of oxidation, Warburg sought a model system in which he could study the process. For this purpose he developed experimental paradigms in which activated charcoal or pyrolised blood (both containing iron as constituents) would catalyze oxidations. It was this model that led him to appreciate more fully the role iron played in his Atmungsferment, as it allowed him to demonstrate again the efficacy of citric and tartaric acids in decreasing the rate of oxidation. As a result of these excitatory and inhibitory studies, Warburg's localization of respiration in his iron-based Atmungsferment seemed assured.

Warburg's work was interrupted by military service during World War I (see Krebs 1981). After the war, Warburg returned to studying the iron-based oxidation enzyme, using charcoal containing iron constituents as a model. He also began to use hydrogen cyanide as a poison. This was significant because hydrogen cyanide behaved differently than narcotics. Unlike narcotics, dosages of hydrogen cyanide that reduced respiration to 50% of normal levels did not inhibit functions such as cell division. The more important fact in establishing the role of an iron-based enzyme in oxidation was that the inhibitory action of hydrogen cyanide could not be

a result of being adsorbed on the catalytic surface. It was adsorbed much more weakly than narcotics. Warburg therefore concluded that the effect of hydrogen cyanide was the result of chemical action between the cyanide and the iron catalyst, which now appeared to function like an enzyme.

Warburg used the charcoal models in two ways. One was to defend the role of his Atmungsferment. By showing that the artificial model behaved like living tissues in oxidizing various amino acids and that it was affected by narcotics and hydrogen cyanide in the same manner as living cells, he gained substantial support for the theory that an iron-based enzyme was the active component in biological oxidation.[15] The charcoal models also gave him a means of studying the catalytic agent. By showing that the charcoal formed by burning sugar in the presence of silicates was inactive—as was similar charcoal formed by adding iron salts before heating—but that adding hemin before heating made the charcoal catalytically active, Warburg established that iron had to be combined with nitrogen to be active.

In 1924 Warburg presented his iron-based model of cellular respiration and proposed a mechanism for its action (cf. Warburg 1925). He proposed that through a reversible change in valency, the iron in the catalyst transported molecules of oxygen to the substrate:

> Molecular oxygen reacts with divalent iron, whereby there results a higher oxidation state of iron. The higher oxidation state reacts with the organic substance with the regeneration of divalent iron. (1924, p. 479)

In this paper Warburg defined an Atmungsferment as "the sum of all catalytically-active iron compounds in the cell" (p. 494). In succeeding years other research showed that a large number of enzymes were involved in cell oxidation; Warburg, however, retained his localizationist view, maintaining that *all* enzymes were components of his Atmungsferment and that laboratory evidence for their individuality was only an artifact of the experimental techniques those investigators employed. The basis of Warburg's argument was that the breakdown of the oxidation process as it occurred with extracted enzymes was different from that in poisoned cells. With extracts different substrates are oxidized, but "[i]f a fraction of the respiration is inhibited by carbon monoxide, the uninhibited respiration is in every respect the same as the inhibited fraction. . . . It follows that all iron atoms which transfer the oxygen in respiration, in one kind of cell, are linked identically" (1930, p. 357). Thus, Warburg was able to maintain the thesis that his Atmungsferment was *the* respiratory enzyme.

One of the problems Warburg faced in developing his model of oxygen activation was that he could not separate and isolate the Atmungsferment

from the cell. This was partly due, he thought, to the low concentration of the enzyme in the cell, and partly to its instability. Thus, he saw the need for an alternative technique for studying the oxygen activating system:

> It seems therefore that the old difficulties of the enzyme problem render advance impossible—the instability of the enzyme and its nearly infinitely low concentration. Only a method which is independent of the quantity of the enzyme and which does not require its separation from the cell can overcome these difficulties. (1930, p. 349)

In the later part of the 1920s Warburg developed an additional research method for studying Atmungsferment that met these requirements: poisoning with carbon monoxide. Carbon monoxide reacted with Atmungsferment much like it reacted with hemoglobin, with the exception that it required a much higher ratio of carbon monoxide to oxygen in order to inhibit oxidation. Warburg (1927) observed that carbon monoxide inhibited the effect of iron. This provided additional confirmation of the importance of the Atmungsferment, but it led to even more significant developments. One of the important facts about carbon monoxide poisoning of hemoglobin is that it is light sensitive. Under the influence of light, carboxyhemoglobin tends to dissociate. Warburg showed that yeast poisoned with carbon monoxide showed a similar response to light. The fact that the Atmungsferment was poisoned by carbon monoxide, and that this poisoning was reversed by light, gave Warburg vital information about the identity of the Atmungsferment; it allowed him to differentiate it from other iron compounds by virtue of their different affinities to carbon monoxide and their different sensitivities to light.[16]

In addition to differentiating the Atmungsferment from other enzymes, Warburg used carbon monoxide to fingerprint the enzyme based on its absorption spectrum. He subjected poisoned tissue to light of different frequencies and tested to see how much the carbon monoxide poisoning was reversed. Warburg (1928, 1929) thus identified the absorption spectrum of the enzyme and showed that, while it resembled that of iron porphyrins such as hemoglobins, it was not identical to any known porphyrin.[17] While the new experiments Warburg developed in the 1920s were elaborate and provided powerful support for the existence of an iron-based component of the cell involved in oxidation, the logic of his argument remained much the same as before. The fact that he could increase or decrease rates of respiration by employing factors that affected this iron agent led him to treat it as *the factor* responsible for cellular respiration. Once again, there was no room for more than one agent, and no tolerance for complex organization.

The Conflict between Wieland and Warburg

The power of direct localization can be underscored by turning to how the principal researchers responded to each others' views. Wieland and Warburg each argued that they had identified the primary mechanism of oxidation and that the mechanism proposed by the other party was either not involved in biological oxidations or was an artifact of the experimental procedures employed. Each investigator had a considerable investment in his own explanation, and their differences were reinforced by disciplinary differences. Even when researchers came to accept the involvement of reactions involving substrate hydrogen and molecular oxygen, both principals refused to acknowledge the value of the contributions of the other and continued to emphasize their differences. It would, however, be overly simplistic to view this conflict as simply one of ego. We think it is also a product of their respective research programs, the reasoning, and the kind of evidence each researcher offered to support his thesis: Each assumed that the task was to find a single, discrete agent. Each relied on inhibitory methods. Each used an artificial model system to provide evidence for his localized mechanism. Artificial models, however, can only show that a process like that proposed *might* occur, but not that it is sufficient, or even that it *does* occur in isolation from any other processes. Wieland and Thunberg, as well as Warburg, did offer evidence to suggest that the modeled process actually occurs in living organisms, but, even if compelling, that does not establish that only that process is operative. Finally, both Wieland and Warburg relied on evidence that if the process they proposed was interrupted, oxidation ceased. Such inhibitory studies, however, can only show that the process is *necessary* to the overall reaction, not that it is *sufficient* to explain it.

To appreciate this point, let us briefly consider the responses Wieland and Warburg each presented to the other. When Wieland confronted Warburg's alternative theory, one focused on oxygen activation, he tied it with that of Engler and Bach, construing the role of ferric oxide in Warburg's scheme as analogous to that of hydrogen peroxide:

> The most generally accepted view is that iron compounds 'fix' molecular oxygen in peroxides of high oxygen potential and liberate it from these in an active form to the substances to be oxidized in the cell. Since iron is able only in the divalent condition to activate oxygen, catalysis by iron of biological processes is only possible where the substrate can reduce the iron in the postulated peroxide to the ferrous state. (1932, p. 24)

Wieland pressed two critical objections. One challenged the generality of Warburg's model; the other threatened its internal adequacy. Thus, Wie-

land pointed out that oxidation by divalent iron could not account for all known biological oxidations, and that the ways of extending the divalent iron theory to cover these cases (by assuming that the iron occurs in complexes) were implausible and ad hoc. He went on to say that the "hypothesis of the activation of oxygen by peroxide formation is *a priori* unsatisfactory, for it is difficult to reconcile the specificity of oxidizing enzymes with such a theory" (ibid., p. 25). Several substances oxidizable in vitro were not oxidizable by living cells—something Wieland thought could not be explained if all that was required for oxidation was an attack by activated oxygen.

Warburg's research made extensive use of hydrogen cyanide as a tool for inhibiting oxidation and as pointing to a special oxygen-activation mechanism. Wieland acknowledged that hydrogen cyanide affected oxidation with oxygen differently than with methylene blue, and this, he says,

> has led to the raising of serious objections to the theory of simple hydrogen activation. On this theory we could not foretell that the activated methylene blue would react practically unaffected by hydrogen cyanide, while the action appears impossible when oxygen is present. Thus it has been argued that the modes of action of the two types of acceptors are different. Quinone and the dyestuff are apparently susceptible to hydrogenation by labile hydrogen while molecular oxygen requires special activation which derives through some particular function of the enzyme. (Ibid., pp. 38–39)

Wieland claimed that this theory required a specialized "co-ferment" to activate the oxygen, but contended that "the assumption of such complicated relationships is unnecessary." He proposed instead to explain the phenomenon by postulating a greater affinity of hydrogen cyanide than oxygen for the active surface of the enzyme. He maintained that such a process could also explain the fact that in different organisms, different hydrogen acceptors were effective (ibid., p. 41).

Wieland took the differences with Warburg seriously, as was evidenced by the fact that the last two of the six chapters in his book (1932) were devoted to the catalytic effect of iron. He asked "whether our knowledge of the catalytic effect of iron in autoxidation may be reconciled with our conclusions drawn on grounds of the dehydrogenation theory, or if the two are entirely irreconcilable" (ibid., p. 86). Wieland contended that the process of oxidation achieved with iron catalysts alone is quite different from what occurs in the cell—a fact he attributed to the multiplicity of enzymes involved in biological oxidation. He then focused his attention on how iron might figure in biological oxidations. He considered two models. The first was Warburg's scheme according to which iron shifted from

a divalent to a trivalent state, thereby activating oxygen, which reacted with the hydrogen from the substrate (B); for example,

$$2FeO + O_2 \Rightarrow Fe_2O_4$$
$$Fe_2O_4 + BH_2 \Rightarrow Fe_2O_3 + H_2O.$$

The alternative scheme invoked a dehydrogenation of a ferrous hydroxide, forming hydrogen peroxide; for example, with oxygen, we have

$$2Fe(OH)_2 + O_2 \Rightarrow 2FeO(OH) + HO\text{–}OH$$
$$HO\text{–}OH + As(OH)_2OH \Rightarrow OAs(OH)_2OH + H_2O.$$

Wieland rejected the first proposal—that is, Warburg's—on the grounds that adding ferric iron at low temperatures had little catalytic effect. Ferrous iron, on the other hand, exerted considerable effect until it was transformed into the ferric state, at which point the reaction slowed dramatically. In the process of transforming ferrous iron, though, the amount of oxygen used in the reaction exceeds the amount theoretically required. Wieland therefore defended the second model, involving ferrous iron catalyzing a dehydrogenation, as in better agreement with the oxygen-consumption data. He proposed that iron functioned in the ferrous hydride by activating the hydrogen. According to this model, the oxidation of the iron did not catalyze the reaction, but actually brought it to an end. Wieland thus tried to show that the iron Warburg claimed was involved in oxygen activation was in fact functioning in hydrogen activation.

Thus, even as late as 1930, Wieland remained dedicated to a single-factor model with direct localization. He viewed Warburg's mechanism as a competitor and as inconsistent with his own. Accordingly, he attacked the specifics of Warburg's model and reinterpreted Warburg's evidence to make it compatible with his own hydrogen-activation model.

Warburg, for his part, viewed Wieland's theory as highly speculative. He prized his own experimental ability and tended to view the work of other researchers as less exacting.[18] At first he simply denied that the dehydrogenation model was empirically viable. In the 1930s, however, his own experimental work led him to accept that oxidation did involve, in part at least, a mechanism for removing hydrogen from substrates. He identified coenzymes that figure in the transport of the removed hydrogen atoms. However, this did not lead to an acceptance of Wieland's earlier work, which he continued to reject as simply speculative chemistry:

> The oxidation theory of Wieland, no less than the opposing oxidation theories of Engler and Bach, were premature because when they were proposed nothing was known about the chemical constitution of the ferments participating in the respiration. They were theories regarding the mechanism of chemical reactions,

> proposed without knowledge of the participants in the reaction. Such theories cannot be other than erroneous, and they must disappear to the extent that the chemical nature of the reaction partners—in this case the chemical constitution of the ferments—is elucidated. (Warburg and Christian 1933, p. 405)

Warburg also pressed much more specific objections to Wieland's model. As we have seen, he contended that the ability of hydrogen cyanide to inhibit cellular oxidation discredited Wieland's theory. He reasoned that, if the mechanism were hydrogen activation, there would be no process for hydrogen cyanide to inhibit.[19] Here Warburg's commitment to a single mechanism seems particularly evident.

Warburg's response to another objection brought by Wieland is also illustrative of his commitment to direct localization. Wieland's objection focused on the occurrence of auto-oxidizable reactions that did *not* involve heavy metals such as iron. Warburg countered by showing that many of these reactions did in fact involve minute quantities of iron or other heavy metals. Thus, cysteine appeared to be auto-oxidizable, but Warburg established that both hydrogen cyanide and pyrophosphate could inhibit the auto-oxidation of cysteine, a result difficult to explain on any other grounds than that a heavy-metal catalyst was involved. Warburg then developed techniques for removing traces of iron from cysteine compounds and showed that once cysteine was purified, it became less auto-oxidizable (1927; for additional examples, see Warburg 1949, ch. 5). Warburg was, thus, committed to showing that only one factor was causally responsible for respiration and to establishing this factor's identity.

4. Conclusion: Direct Localization And Competing Mechanisms

In the previous chapter we emphasized the identification of a locus of control and the assumptions it involves. We have treated this as an application of two heuristics, decomposition and localization: we decompose the activities of nature and localize some of them in a particular system. Often a single system becomes a locus of control for a variety of activities, and the explanatory task becomes one of explaining this variety. Having identified a system as a locus of control, we treat it as a complex of components that are again subject to decomposition, and we localize systemic functions within components. It is then an empirical matter to inquire whether the behavior of the system as a whole can be explained in terms of the behavior of the identified component systems, and what kind of organization is needed to do so. The brief flowchart presented at the end of the previous chapter can thus be supplemented as in Figure 4.2.

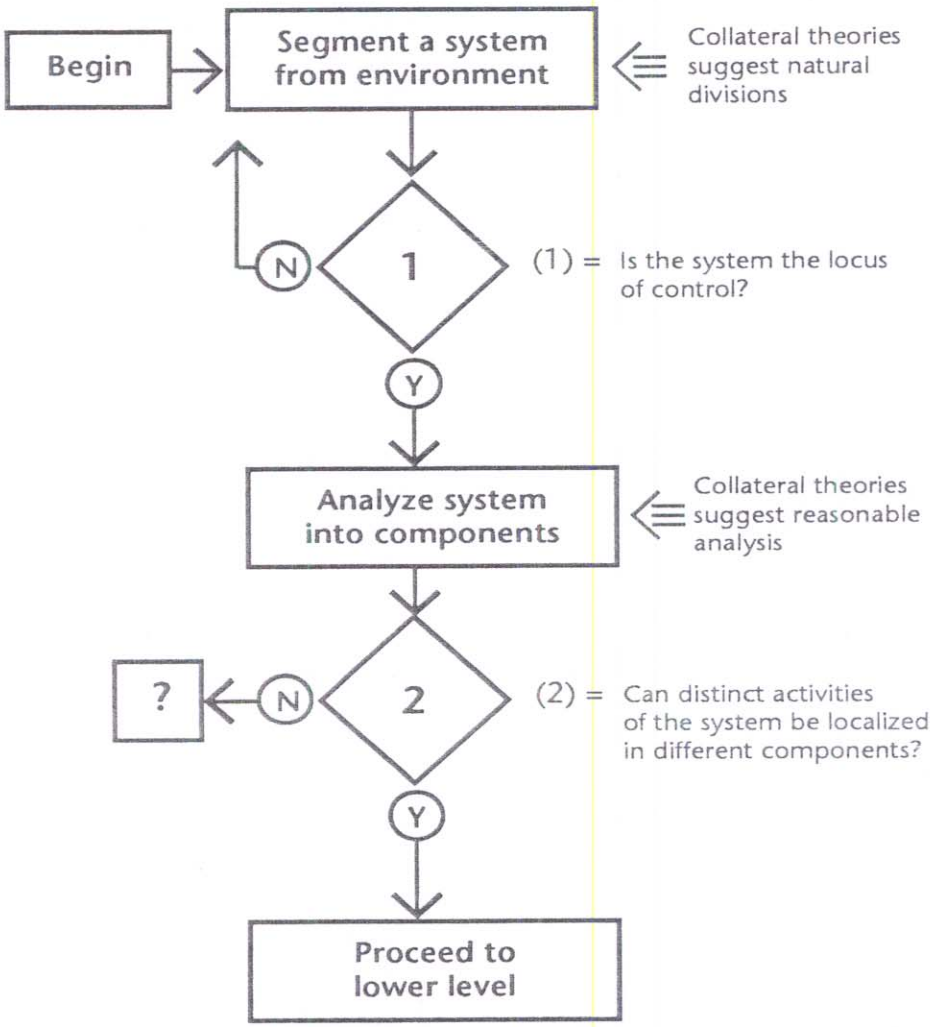

Figure 4.2. The Second Choice-Point. Having identified a system as a unit responsible for some function, one subsequent step involves decomposing the system, treating it as a complex of components, and localizing responsibility for systemic functions in specialized components. Localization and decomposition are often strongly influenced by collateral theories.

Direct localization depends critically on the ability to analyze the system into subsystems that operate in relative independence of one another. This analysis can be structural or functional. Gall and his followers employed a structural analysis of the brain and a functional decomposition of behavior: the mind was conceived as a system of discrete faculties; the brain as a system of discrete physical organs, each with a characteristic capacity. The analysis of the system need not be structural, though it is often implicitly supposed that it could be structurally analyzed even when the operative criterion is functional. Wieland and Warburg focused on identifying the agent of intracellular oxidation. Neither researcher had the

physical information required to conduct a structural analysis into physical units or to provide a structural analysis of the enzymes involved. Warburg did postulate physical structures (which later were equated with mitochondria) as the surfaces on which respiration was effected, but these did not play any significant role as his model unfolded and developed. Warburg and Wieland thus relied on a functional decomposition of the system.

The choice between structural and functional analysis was guided by available theoretical considerations—collateral theories—and practical feasibility. Gall thought that a functional decomposition of mental capacities was afforded by his identification of different traits and capacities. The structural analysis into organs of the brain was provided by what appeared, at a morphological level, to be reasonably differentiated units of the brain. For Warburg and Wieland the functional subsystems were inferred from the way in which the overall respiratory process was conceived. Since hydrogen and oxygen did not interact under the general kinds of conditions prevailing in the cell, one or the other had to be activated to facilitate the reaction. The functional characterization of the systems was a natural outgrowth of the characterization of the problem, and this was a consequence of the theoretical framework in terms of which the problem of biological oxidation was posed.[20]

Given an analysis into constituent subsystems, the next problem is the identification of a likely component for producing a particular behavior of the system. If the system is analyzed into structural subsystems, then the natural problem is one of finding features of various structures that suggest that the structure will have the capacity in question. If the subsystems are differentiated functionally, then the functional decomposition will already have defined the subsystems in question, and the problem will be to isolate an appropriate structure. For Warburg and Wieland the relevant subsystems were already defined by the assumption that either hydrogen or oxygen had to be activated in order to react with the other. Whether one begins functionally or structurally the approaches are not incompatible, and, in general, the only satisfactory solution will be one that reflects both functional and structural properties.

Identifying an activity of a system with the activity of a component subsystem presupposes that the component structure will provide an adequate explanation of the system's capacities. Direct localization depends on demonstrating the *sufficiency* of the mechanisms thus isolated. We have noted a number of experimental strategies researchers deployed in garnering evidence relevant to this issue. The simplest cases rely on correlations between subsystems and the behavior of the system as a whole. According to Gall's view, the critical correlation was between the development of psychological faculties, as observed in behavior, and the size of the brain center in which the faculties are localized, as evidenced by cra-

nial prominences. Positive evidence for direct localization was reflected in a high correlation. As we will see in Chapter 6, Broca subsequently introduced a novel and more compelling type of evidence for localization by correlating damage to a region with behavioral deficits. Given the assumption that faculties are localized in specialized regions, the correlation of deficits with behavioral irregularities provides strong evidence for particular localization claims. Broca had to rely on natural deficits, and that left open the question whether the correlation was accidental or was revealing of causal structure.

In the case of cellular respiration, researchers were able to introduce experimental techniques to interrupt or excite the localized center and thereby demonstrate the effects such changes had on system function. If an effect was still present after a component was removed, that provided strong evidence that the component did not play a critical role. Wieland's success in obtaining oxidations in the absence of oxygen afforded what he thought was compelling evidence that oxygen activation was not the critical component in oxidation. On the other hand, if removing a component did disrupt the function, that was good evidence that the component did play a central role. When Warburg found that cyanide inhibited cellular respiration, and he then provided reason to think that it acted by binding with cellular iron, he saw this as indicating that his iron-based oxygen-activating enzyme was critical to oxidation and cellular respiration. Excitation and inhibition can play important experimental roles by providing positive or negative evidence: Warburg found that adding iron salts to a cell facilitated oxidation, and he took the result to indicate that iron played a critical role in respiration; Wieland subsequently used the failure of ferric iron to induce catalysis as evidence against Warburg's model.

The success of direct localization will, of course, vary. In some cases the evidence may be compelling in favor of the view that a particular component is in fact responsible for the behavior of the system under study. Direct localization is not sufficient to describe a mechanism that can explain how a function is performed; it serves only to localize the "center" in which the function is performed, leaving the residual problem of explaining *how* the component performs the function in question. To explain subsystem operation, the next move would be to redirect inquiry to a yet lower level, possibly reapplying the kinds of strategies we have illustrated. We will discuss an example of this sort of theory development in Chapter 6.

It is important to see that the level of scientific debate is often over *which* component is responsible, and not over *whether* there is some isolated component. Wieland and Warburg were not arguing for decomposability or localization per se, but rather over the specific localization that was to prevail. Assuming there is a simple, direct localization is tanta-

mount to assuming the system has a simple form of organization; it presupposes that there is relative independence of the component subsystems and correspondingly that the organization is either aggregative or composite. Even when this is correct, it may be true only to a first approximation. When the problem is one of explaining some particular dimension of a system's behavior, a localized component may explain variations only to a first-order approximation. Other components may turn out to be relevant. Once we have accounted for primary variations in the behavior of a system in terms of one component or a set of components, it is possible to focus on second-order interactions. In other cases, as with biological oxidation, there may be multiple components involved at the same level of organization. The task then becomes one of discovering the interactions between those components and showing how, together, they constitute an appropriate mechanism. The cases examined in Chapters 7 and 8 explore this strategy.

If we become convinced that no direct localization can be correct, even as a first approximation, and that we cannot attribute the behavior of a system to any one component, there are a variety of options. One is the path followed in the case of cell respiration. We may accept that more than one component is involved in producing the behavior and seek a systematic account of the components' interaction. In other cases, however, researchers have opted to reject decomposition and localization altogether, and consequently abandoned the search for mechanisms. It is to such cases that we turn in the next chapter.