Reprinted from *New Beer in an Old Bottle: Eduard Buchner and the Growth of Biochemical Knowledge,* pp. 67–122, ed. A. Cornish-Bowden, Universitat de València, Valencia, Spain, 1997

# FROM FRIEDRICH WÖHLER'S URINE TO EDUARD BUCHNER'S ALCOHOL

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The *callidum innatum,* the vital flame, or animal spirit in man, is supposed the cause of all motions in the several parts of his body… This pure spirit or invisible fire is ever ready to exert and shew itself in its effects…, cherishing, heating, fermenting, dissolving, shining, and operating, in various manners…

BISHOP BERKELEY (1744): *Siris,* sections 156, 157.

Where now the vital energy, that moved
While summer was, the pure and subtle lymph
Through the imperceptible meandering veins
of leaf and flower?

WILLIAM COWPER (1784): *The Task,* Book VI, 134–137

"I don't know what you mean by 'glory'" Alice said. Humpty Dumpty smiled contemptuously. "Of course you don't — till I tell you. I meant 'there's a nice knock-down argument for you!'"

"But glory doesn't mean 'a nice knock-down argument,'" Alice objected.

"When *I* use a word," Humpty Dumpty said, in a rather scornful tone, "it means just what I choose it to mean — neither more nor less."

"The question is," said Alice, "whether you can make words mean so many different things."

"The question is," said Humpty Dumpty, "which is to be

> master — that's all."
>
> LEWIS CARROLL, *Through the Looking Glass*

> The words and phrases used by men and women throughout the ages are the loveliest flowers of humanity… the whole past from the time when the word was coined is crystallized in it; it represents not only clear ideas, but endless ambiguities.
>
> GEORGE SARTON (1952) *A History of Science,* Preface

> When people refer to non-enzymic reactions as being "chemical" they give the impression that the enzymic reaction does not belong to chemistry
>
> DUILIO ARIGONI (1994)

A scientific discovery is both culmination and promise. It is a child of its times and a progenitor of the future. The greater the discovery, the greater the number of threads that it weaves together into a new fabric of coherence and understanding, the greater the tapestry of promise that it unfolds. Like a work of art we admire it, celebrate it in its own right. It has been said of Johann Sebastian Bach that he is "a Janus, one of whose faces looks at the past, the other to the future" (Cart, 1885). On 22 February 1828, Friedrich Wöhler, who had just shown that urea can be produced from ammonium cyanate, wrote a letter to his mentor, Jöns Jacob Berzelius in Stockholm:

> I can no longer, as it were, hold back my chemical urine; and I have to let out that I can make urea without needing a kidney, whether of man or dog.
>
> KEEN (1976); WALLACH(1901)

Both Wöhler and Buchner occupy Janus-like positions in the development of different branches of chemistry. We are here concerned with tracing the development of biological and what we now call biochemical ideas and experiments that led to Buchner's accomplishment of cell-free fermentation. We must try to understand his work not only, as is usually done, as originating beam but also as gathering focus: the one clarifies the other. At the end of this article we will return to Friedrich Wöhler, for at that point an enquiry into divergencies and coalescences in the historical development of organic chemistry and of biochemistry will be extremely instructive. For the present, and for the bulk of this article, however, we must focus on topics of more narrowly defined biological interest.

The last quarter of the 18th century had witnessed a remarkable dissolution of ancient shackles expressed in terms such as *anima, entelechy, soul, archeus, vital force* that, in the words of Joseph Needham (1956; quoted by Teich, 1965)

> dance processionally through the history of European thinking because a *deus* always had to be found for a *machina.*

Lavoisier (1777) had shown by quantitative measurements that "the respiration of animals [and] combustion [are] operations... much more closely related than is obvious at first sight." The Abbott Lazzaro Spallanzani (1780), continuing work by René Antoine Ferchault, Sieur de Reaumur (1761), had found that gastric digestion is a chemical activity that can continue outside the organism: the living system was demonstrated not to be needed for the continuation of a biological function. These were experimental results whose insistence chipped away at an edifice of vitalist supposition that held earlier science in its thrall, but they only chipped away.

Our story continues with meanderings through intermeshing central threads of 19th century discovery and 19th century contention, each with its own antecedents, that occupied some of the greatest minds of 19th century science: fermentation, digestion, vital force, the development of organic chemistry, catalysis, enzymes. Among these, it is vital force that provides a recurring leitmotif that informs curiosity, inflames contention and serves as a convenient, ever available refuge of explanation. Vitalist ideas encompass a wide range of biological contention throughout the 19th century and into our own century. We will be concerned with the saga of a fencing match between vitalism and biochemical observation, where believers in vital force, driven ever more into regions of decreasing application, continue to maintain claims of validity in rearguard actions of persisting subtlety, while its opponents, fed more and more by chemical verification, dispel vital force insistence with ever more encompassing demonstrations of irrelevance.

Buchner's discovery of cell-free fermentation modified perspectives and, in so doing, as always in good science, it raised major new issues that were, in turn, to capture the attention of some of the most eminent biochemists of the 20th century: metabolic pathways, controls, structural correlations at the molecular and cellular level, compartmentation, channelling, biochemical unity, eukaryotic compared with prokaryotic gene expression, and many more.

Let us begin with the remarkable thoughts of one Johann Christian Reil, clinical professor and director of the clinical institute at the University of Halle, who in 1795 founded the *Archiv für die Physiologie,* the first journal devoted exclusively to physiology. The very first article in volume 1 of that journal is entitled *Von der Lebenskraft* ("About the Vital Force"). What a topic, we would say, to begin a scientific journal! What a reflection of the scientific preoccupation of its times! But Reil had his own message. In this article he argues elegantly, mainly from logical considerations, that no vital force can exist, and that the phenomena of life can and should be explained in terms of chemistry. Reil was a famous physician — among his patients were Goethe and one of the brothers Grimm (Eulner, 1976; Wallach) —, a "medical educator… and… innovator in psychiatric care" (Risse, 1975). He is remembered by anatomists for the limiting sulcus of Reil and for the island of Reil. His article on the vital force still makes for fascinating reading. Space permits only a few excerpts:

> Medical doctors and philosophers have always been inclined to deduce the phenomena of living nature… from ghosts… The ancients assumed nymphs in the trees, van Helmont an archeus, and Stahl a soul as principle of the appearance of living beings. However, experience gives no proof for the existence of ghosts… Must we deduce the magnetic property of iron as due to something else than matter since magnetic phenomena are not found associated with tin, stones or wood?… A special name is used for the formation of the substance of living beings, because of their remarkable perfection. Organ and organization hence is the formation and structure of living bodies. Linguistic usage and the derivation of the word *organ* teaches that the word *organization* refers to the formation of this substance. It follows that one has used the word *organization* metaphorically, i.e. one has named living beings, simply because of one of their properties, as organic beings… We have here a reason that so many errors and misunderstandings have crept in relation to the meaning of the word *organization* … All phenomena in the world of bodies are results of a given form and mixture of matter… Force is something that cannot be separated from matter, it is a property of matter, and it is only through matter that it can

> be manifest… [We] would not need the concept of force, which leads to several erroneous consequences… I have used the term *vital force* for the force of matter that characterizes the plant and animal kingdom… Perhaps others will find the term *organic force* to be more suitable. However, I did not use this term since the word *organization* by common usage denotes the formation of living beings. Words… are arbitrary symbols of our concepts, and it… is important that the concept associated with a word be precisely ascertained… The physical, chemical and mechanical forces of animal bodies are, as one says, subordinated to the vital force… However, such a domination and subordination can actually not be accepted in nature… Our subjective concepts, which we import into nature, frequently dazzle the understanding of dumb people and provide them with a toy instead of with reality… If conditions change, then laws are changed not in nature but in our understanding… [Erasmus] Darwin is of the opinion that growth and the maintenance of living beings occurs not via chemical affinities, but via animal appetites. Every part, he says, has its own appetite… However, can one possibly think of an appetite without any supposition? If, indeed, we remove those suppositions from Darwin's animal appetites, what remains? In fact nothing remains but chemical attraction, unless we wish to denote one thing with two types of words… [The] phenomena of [animal] bodies are activities and properties of their matter.
>
> REIL (1795, my translation)

The term *vital force* was very widely used at the time: Joachim Dietrich Brandis (1762–1845) had written a book (published 1795) with the title *Versuch über die Lebenskraft* ("Experiment concerning the Vital Force"), reviewed by Reil in that first volume of his journal, while the *Grundzüge der Lehre von der Lebenskraft* ("Fundamentals of the Teachings about the Vital Force") of Theodor Georg August Roose (1771–1803) underwent two editions (1779, 1803) and a translation into Italian (1802). In 1794 Alexander von Humboldt used the term, and he is strongly attacked by Reil in the present essay, since Humboldt maintains that the vital force not only does away with the chemical bonds based on chemical affinities, but that it places other obstacles in the way of chemical reactions in living bodies. At the very same time (1795), Humboldt wrote a short story *Die Lebenskraft oder der Rhodische*

*Genius* ("The Vital Force or the Rhodian Genius"), published by no less a person than Friedrich von Schiller in his short-lived monthly journal (1795–1797) *Die Horen.* Reil's long paper, perhaps bolstered by the monumental experimental observations of the likes of Lavoisier, Reaumur and Spallanzani, made short shrift of speculation and would, from our perspective of 200 years later, have been expected to sound the death-knell of vitalist thought.

Yet this was not to be. Earlier ghosts returned with clinging insistence. It is possible that Reil's work was rather too philosophical, and it has been stated, in contrast, that the clear data of Reaumur and Spallanzani were not accepted by the scientific world of the period (Effront, 1917, p. 15), perhaps because they "are overwhelmingly experimental with almost no polemics and very few theoretical considerations" (Bates, 1962, p. 360).

Fifteen years later (1810) we encounter an address *Progress and Present State of Animal Chemistry* given by the eminent chemist Jöns Jacob Berzelius on the occasion of the completion of his term as President of the Royal Academy of Sciences, Stockholm:

> We can regard the whole animal body as a machine which gathers materials for ceaseless chemical processes out of the food that it receives,… but the cause of most of the phenomena in the animal body is so deeply hidden from our understanding that we will certainly never discover it. We call this hidden cause the *vital force…* The chain of our experiences must always end in something beyond understanding, but unfortunately this incomprehensible something plays the main role in animal chemistry and in this manner influences every process, even the smallest one. It follows that we can at the most learn about the nature of the products, while the manner in which these are formed must remain an eternal secret.

This address must have generated a great deal of interest, for within three years it had been published in English and in German (Berzelius, 1812; Simmer, 1955, p. 219; Rocke, 1992, p. 121).

Berzelius's views on the inscrutable nature of living processes did not long remain unchallenged. In a direct attack, 1815, on Berzelius's so-called zoochemistry, one of Reil's students, Georg Karl Ludwig Sigwart, gives his own view of the content of zoochemistry. He emphasizes chemistry and, in contrast to Berzelius, completely ignores the vital force. He gives a definition of zoochemistry that would apply to large parts of modern biochemistry:

> Animal organisms have chemical activities that depend on life and on organization. We use the term *chemical life processes* to denote these activities… [These] chemical activities of the living organisms, or the animal-chemical life process, constitute the topic of zoochemistry which studies (1) the products of these chemical life processes, and (2) modes of formation, the genesis, of these products from the perspective of [*in Absicht auf*] the chemical process.
>
> SIGWART (1815), SIMMER (1955, my translation)

Berzelius's views, however, prevailed. In 1827 a German edition of his highly influential *Textbook of Chemistry* was published. The section on organic chemistry starts with the following sentences:

> In living nature the elements appear to obey quite different laws from those in dead nature. Hence quite different products result from their various interactions than in inorganic nature. The discovery of the cause of this difference between the behaviour of the elements of dead nature and that of living bodies would be the key to the theory of organic chemistry. This cause is, however, so hidden that, at last for the moment, we cannot hope to find out what it is… The multitude of chemical processes [in a living body]… eventually stop, and from this moment the elements of the body that had been alive begin to obey the laws of inorganic nature… The being of the living body is… based not in its inorganic elements but in something else… The something we call the *vital force*…
>
> (My translation)

This passage persisted virtually unchanged through many editions, including the posthumous fifth edition (1856) (Teich, 1965). Berzelius's views, however, were inconsistent at best. Already in 1831 he expressed the view that "We have no right to contemplate other than chemical forces at work in living bodies" (quoted by Lindroth, 1992), while in 1838, even more forcefully, he viewed "A separate vital force is an unnecessary and detrimental assumption; organic processes obey the same laws as inorganic ones" (see Lindroth, 1992). A detailed analysis of Berzelius's views on vitalism was made by Rocke (1992). His view on the vital force was the subject of the President's address at the 150th anniversary of the Swedish Medical Society by the eminent enzymologist Hugo Theorell (1958). This is not available in any translation. Our story will show that vitalism, hydra-like, had to suffer many slouching, but always incomplete retreats, often as the result of attacks from unanticipated sources.

Berzelius makes another appearance in our story as one-man author of what we would now call Annual Reviews, the celebrated *Årsberättelser öfver Vetenskapernas Framsteg,* or *Annual Survey of Progress in the Sciences,* that he wrote for almost 30 years. In his 15th Annual Review, submitted 31 March 1835 to the Royal Academy, he invents and defines the word *catalysis*. He refers to it as an "only rarely observed force which is probably active in the formation of organic substances." Many examples are given, both of inorganic catalysis and of organic catalysis.

As stated by Malcolm Dixon (1971), "it is important to realize that it was very largely the action of enzymes that gave rise to the idea of catalysis, not the converse as is often assumed". Moreover, catalysis was right from the beginning tied to fermentation. Berzelius discusses fermentation as an example of catalysis:

> This property was not an isolated, exceptional behaviour but proved to be a more general one, exhibited by substances to varying extents… We have found, for instance, that the conversion of sugar to carbon dioxide and alcohol, which occurs in fermentation through the influence of an insoluble substance known by the name of ferment… could not be explained by a chemical reaction between sugar and ferment resembling double decomposition. However, when compared with phenomena known in inorganic Nature, the preceding phenomenon most closely resembles the decomposition of hydrogen peroxide under the influence of platinum, silver or fibrin; it was hence very natural to imagine an analogous activity in the case of the ferment.
>
> (My translation)

What is the relation between all of this and vital force? We will see that the idea of a vital force constitutes a background, a not always expressly stated drone, to a great deal of 19th century thinking about the nature of catalysis and about ideas on the relation between catalysis and fermentation. Catalysis and fermentation dance like a two-step, at times contentious, at times distant, at times partly resolved but never completely harmonious, through the landscape of vitalist contention, till they meet in an apex of resolution in Buchner's work, some 60 years after the term catalysis was invented. Berzelius in his

Annual Surveys "delivered his Olympian judgments on the science of his day" (Lindroth, 1992). At the time that he coined the word catalysis he was, in the words of Arthur Harden (1932, p. 7) "the arbiter and dictator of the chemical world". Twenty five years had passed since his address on the progress of animal chemistry. Clearly Berzelius was fond of a varied armamentarium of mysterious forces, applied both to life and to catalysis. It may not be a coincidence that his catalytic force appeared more or less at the same time that his fondness for a vital force had waned. It has been pointed out "that by making no distinction between his catalytic force in animate and inanimate material… Berzelius expresses an approach that was hardly compatible with the ideas of a vital force" (Hoffmann-Ostenhof, 1987) and that the recognition of the catalytic force in the living body as well as in inorganic nature was "nothing mystical… and was in principle as commonplace as electricity or magnetism" (Lindroth, 1992). In any case, as we shall see, Berzelius's views, influential as they were, did not suffice to abolish the idea of a vital force.

The word *ferment* as used by Berzelius denoted a vaguely definable something that exhibited what he viewed as just one example of catalytic activity, namely fermentation. Many substances, organic and inorganic, were catalysts but were obviously not *ferment*. The word *ferment* was soon to encompass a much wider and much more confused meaning. It is important to note that Berzelius's introduction of the term *catalysis* and its relation to *ferment* coincides almost exactly with the demonstration, a mere two years later (1837) by three independent investigators, Charles Cagniard-Latour, Theodor Schwann and Friedrich Traugott Kützing, that the ferment, yeast, is actually a living organism. However, and here, as so often in this story, the facts of history must be substituted for the intimations of logic, Berzelius, who at the time had rejected his adherence to a vital force, resolutely rejected the notion of yeast as a living entity, and changed his mind only in 1848, the last year of his life, in his last *Annual Survey*. With his acceptance of the living nature of yeast he was one of "a powerful minority" (Harden, 1932, p. 9).

The mid-19th century notion of ferment was subject to confusion since the term was applied both to certain organisms and to certain processes associated with or obtainable from organisms. This ambiguity has a historical basis. The term *ferment* had been used for an agent that, depending on one's preference or need, produces alcohol in accord with an age-old human fascination (cf. Vallee, 1997) or carbon dioxide, as in the 1420 quotation "Use this ferment for musty bred" (*A New English Dictionary,* 1901). The term continued to be used for the recently demonstrated living organism. At the same time the term was used for the few catalytically active substances that could be extracted from yeast and from other sources. This latter use survived, presumably, to retain the initial connection to nonliving substances. Words hide meanings as much as they lead to or express meanings. The multiplicity of meanings of the word ferment summarizes the confusion between process and the basis of process, between chemical reaction and the ununderstood or misunderstood chemical and biological basis of reaction. Vitalism was an expression of this confusion since it provided a convenient refuge or explanation for the circumstance, accidental as it turned out, that process appeared to be indissociable from life. Although a catalytic process was recognized by Berzelius, just before yeast was shown to be alive, to be associated with fermentation, a tortuous logic, if not by him, then as we shall see by others, dictated the presumed catalytic process of fermentation, postulated to be brought about by a catalytic force, to be in turn informed or dictated by a vital force that could only act in an intact organism. It is this kind of thinking that led to solubility or solubilization as the linchpin that decided between the existence or the absence of a vital force. It would have constituted a circular reasoning to think of physicochemical reasons as explanations for insolubility, since this would have been a tacit admission of physics or chemistry as an explanation for an observation or lack of an observation. This kind of intellectual climate had to accept insolubility as a decisive proof of the inability to dissociate chemistry from life. From our perspective of well over a hundred years later it is somewhat difficult to accept the notion that a simple physicochemical distinction should have led to such far-reaching conceptual distinctions that led, as we shall see, not only to the celebrated Berthelot–Pasteur fermentation quarrel, to the invention of the word *enzyme,* but finally to the excitement generated by Eduard Buchner's demonstration of cell-free fermentation.

The highly influential Justus von Liebig by 1839 rejected Berzelius's notion of catalysis and advanced his own ideas about fermentation, based on vibrational energy transfer from yeast to sugar, an idea that resuscitated late 17th century ideas of Georg Ernst Stahl. This theory did not at all depend on an acceptance of yeast as a living being. The notion of yeast as a living organism was considered to detract from the idea of fermentation as a chemical process. Liebig's contemporaries understood his rejection of the participation of living agents in fermentation not only as an affirmation of the commonly held view of this process, but as a veiled attack by the claim by the above three investigators that ferment was indeed a living cell. In the words of Florkin (1972, p. 140):

> Liebig, cock of the walk, who ruled over the whole fabric of the "biochemistry" of the time, had no positive argument to offer and he resorted to a most unpalatable and dishonest procedure (a lesson for those who... believe that the practice of laboratory work develops the ethical sense of the scientist).

In 1839, the good friends Wöhler and Liebig (see Delbrück and Schrohe, 1904) had published in the international journal that they edited with some others (*Annalen der Pharmacie,* later called *Liebig's Annalen),* an anonymous scathing attack on the notion that fermentative activity could possibly be due to a living cell (Wöhler and Liebig, 1839). This attack was felt particularly strongly by the young and sensitive Theodor Schwann, 29 years old at the time, and spelled the virtual end of his remarkably creative and versatile career. It contributed to send him that very same year into exile from Germany to a professorship in Belgium (Florkin, 1972, p. 141; 1975, p. 244) where he accomplished but little in the remaining forty-three years of his life. Liebig as late as the 1851 edition of his celebrated letters (*Chemische Briefe*) (Letter XV) does not mention that yeast is a living organism (Harden, 1932, p. 10). The eminent chemist Charles Gerhardt in his *Traité de Chimie Organique* (1856) resolutely rejected the vegetable matter of yeast (Harden, 1932, p. 10). He stated that

> Evidently Liebig's theory alone explains all the phenomena in the most complete and the most logical fashion. All good minds [*bons esprits*] cannot but rally behind it.
>
> translated from HARDEN (1932, p. 11)

There are various reasons for this rejection of scientific observations or, at times, for argumentation against them. A general reason has been given by Max Planck: scientists never change their minds, but eventually they die (see Northrop, 1961, Mitchell, 1979). There is a general fear that the acceptance of new findings may overthrow current ideas and with them one's own pet artifice which may stand in danger, often unjustified danger, of being exposed as a house of cards. In our case a reason that applies specifically to the rejection by many of yeast as (i) a living agent (ii) responsible for alcoholic fermentation, has been elegantly stated by Harden (1932, p. 9):

> There seems little doubt that both Berzelius and Liebig in

> their scornful rejection of the results of Cagniard-Latour, Schwann, and Kützing, were influenced, perhaps almost unconsciously, by a desire to avoid seeing an important chemical change relegated to the domain of that vital force from beneath the sway of which a large part of organic chemistry had just been rescued by Wöhler's brilliant synthetical production of urea.

There is a remarkable twenty-year gap between the demonstration of yeast as a living organism and further research into the nature of fermentation. In 1857 Pasteur started his work, not with "an alcoholic ferment" but with "a particular ferment, a lactic yeast" (Pasteur 1857, quoted by Fruton, 1972) which he had discovered (Harden, 1932, p. 11), and showed that this ferment was (i) a living organism and (ii) the active cause of the production of lactic acid (Harden, 1932, p. 11). Pasteur then extended this approach to alcoholic fermentation and demonstrated clearly that the process required the presence of yeast. Alcoholic fermentation was concluded to be "a phenomenon correlative with a vital act". It must be emphasized that Pasteur, trained as a chemist, and a strict adherent to experimental facts, was rather more tolerant of the possible chemical nature of the fermentative process than is generally assumed. Thus in his detailed 1860 report on alcoholic fermentation he stated:

> Now, what does the chemical act of the cleavage of sugar represent for me, and what is its ultimate cause? I confess that I am completely ignorant of it… Will one say… that the yeast produces, during its development, a substance such as pepsin, which acts on the sugar and disappears when that is exhausted, since one finds no such substance in the liquids? I have no reply on the subject of these hypotheses. I do not accept them or reject them, and wish to constrain myself always not to go beyond the facts. And the facts only tell me that all the fermentations properly designated as such are correlative with physiological phenomena.
>
> PASTEUR (1860a, pp. 359–360), cf. FRUTON (1972, pp. 55–56)

Pasteur's views were strengthened by the negative results of Friedrich Wilhelm Lüdersdorff (1846) who had been unable to find any carbon dioxide formation after a thorough trituration of yeast for a whole hour between ground glass plates and who concluded that the intact yeast structure, that is to say its living form, was therefore necessary to exhibit its chemical activity. This experiment was repeated by Carl Schmidt in Liebig's laboratory. He ground yeast for six hours (!), again with negative results. Pasteur had two other telling points: (i) the products of many biological transformations showed optical activity, and (ii) alcoholic fermentation was accompanied by the formation of variable amounts of other products such as glycerol, succinic acid and other substances. As to the first observation, Pasteur argued that

> artificial products do not have any molecular dissymmetry… I could not indicate the existence of a more profound separation between the products born under the influence of life and all the others.
>
> PASTEUR (1860b, p. 33), cf. FRUTON (1972, p. 53)

As to the second objection, it was clear that one could not possibly write a simple chemical equation to account for these observations, and it was concluded that something else than simple chemistry had to be at work.

We have up to this point encountered three different theories of the nature of alcoholic fermentation: Berzelius's that regarded yeast as a catalyst, Liebig's that couched the process in terms of chemical vibrations transmitted from the yeast to sugar, and Pasteur's who advanced no mechanism, but, keeping close to the available experimental facts, espoused a strictly organismic view of fermentation. A fourth view was advanced, by the eminent chemist Pierre Eugène Marcellin Berthelot. In 1860, at the precise time that Pasteur advanced his fermentation ideas, Berthelot found that cold-macerated yeast upon exposure to pressure, followed by digestion with water and filtration, yielded a solution that, in his words

> inverted the sugar in the same way as yeast itself… Yeast extract therefore contains a specific [*particulier*] ferment, soluble in water and able to convert cane sugar to invert sugar… On the basis of [these] new experiments… I think that this plant [i.e. yeast] acts on sugar not because of physiological activity, but simply by means of the ferments that it has the property of secreting, in the same way as germinating barley secretes diastase, almonds secret emulsin, the pancreas of an animal secretes pancreatin, and the stomach of the same animal secretes pepsin. Among the secreted ferments, those that are soluble can be isolated and purified up to a certain point… The same is true for the glucosidic

> ferment, which is one of those that beer yeast contains. On the other hand, the insoluble ferments remain entangled in the organized tissue and cannot be separated from them. In short… it is seen clearly that the living being is not the ferment; but it gives rise to it. Also, once the soluble ferments are produced, they act independently of any further vital act; this activity shows no necessary correlation with any physiological phenomenon. I insist on these words in order not to leave any ambiguity about my way of regarding the action of soluble ferments… If a deeper study leads to an extension of the viewpoint that I propose and to its firm application to the insoluble ferments, then all fermentations will be found to lead back to one and the same general concept and it will [then] be possible to study [the insoluble ferments] under the same heading as the activities due to contact with acids and [like] chemical agents properly so called.
>
> BERTHELOT (1860a), my translation

In his influential textbook of organic chemistry of the same year (1860) Berthelot adds a few phrases and continues, significantly,

> To banish life from all explanations relating to organic chemistry, that is the aim of our studies. Only thus will we succeed in building a science that is complete and that can exist by itself, that is to say, one that can be used efficiently to understand physiological changes and their artificial reproduction.
>
> BERTHELOT (1860b), my translation

Remarkable words indeed!

The 1870s provide a rich and often rewarding field for present-day treasure hunters of early insights into the nature of fermentation. Many eminent scientists were attracted. The views of only a few can be considered here as they parade with a pedlar's insistence through the landscape of 19th century contention. Claude Bernard (posthumously), Moritz Traube, Felix Hoppe-Seyler agreed with Berthelot and not with Pasteur. Pasteur, in the words of Fruton (1972, p. 53), "was endowed with an exceptional ability to attack controversial problems by selecting for critical examination the weak points in a theory he intended to disprove". And then there was Liebig, still influential and unwilling to change his mind. In 1870, now 70 years old, he advanced a

> rambling attack… on Pasteur's experimental evidence. Liebig had by then more or less come around to the view that "ferment" was an enzyme, not a decomposing protein. But he clung enough to his older outmoded view to make him a sitting duck, and in 1872 Pasteur published a brief and devastating rebuttal to which Liebig never replied and which, according to legend [see Volhard, 1909] hastened his way to the grave.
>
> KOHLER (1971, pp. 38–39)

Some other events occurred in the same decade that bear directly on our story: Marie Manasseïn (1871) claimed to have obtained an extract from yeast that brought about alcoholic fermentation; this paper attracted very little attention. In 1876 the word enzyme was coined by W. F. Kühne, and from 1876 to 1879 Berthelot and Pasteur engaged in their famous so-called fermentation quarrel.

First Claude Bernard as an example of the whirlpool of ideas for and against vitalist notions. Bernard was not directly concerned with alcoholic fermentation but his ideas need to be mentioned, since they figure prominently in the celebrated Berthelot–Pasteur fermentation quarrel, discussed below. Bernard resorted to the notion of vital force when he distinguished the breakdown of glycogen in the liver as being an

> entirely chemical action, which can be effected outside the influence of life… [while the formation of glycogen was] an entirely vital action… because it is not effected outside the influence of life.
>
> BERNARD (1857), quoted by FRUTON (1972, p. 407)

In his last book (1878–1879) Bernard again distinguished between

> phenomena of *vital creation* or *organizing synthesis* [and] phenomena of death or o*rganic destruction,* [the former being] truly vital, [the latter] of a physico-chemical order… comparable to a large number of chemical decompositions or cleavages… It is worthy of note that we are… the victims of a habitual illusion, and when we begin to designate the phenomena of *life,* we in fact indicate the phenomena of *death.*
>
> quoted by FRUTON (1972, pp. 60–61; italics in the original)

We find here a fascinating abdication of vitalist ideas in experimentally verifiable areas, and a stubborn preservation of these ideas in regions where theory held sway over observation. While Berthelot leaned strongly to the chemical side of living processes, Bernard clung to vitalist attitudes in regions where chemical experimental evidence had not yet usurped vitalist preferences. Although Bernard retained as much of vitalist notions as he thought the evidence allowed, his acceptance of a chemical view of degradative processes was strongly attacked posthumously by Pasteur (1879; Fruton, 1972, p. 62).

Around 1872 Hoppe-Seyler addressed the phenomenon of alcoholic fermentation:

> The only question to be determined is whether [the] hypothesis is too bold which assumes that in the organism of yeasts there is a substance that decomposes sugar into alcohol and $CO_2$... I hold the hypothesis to be necessary because fermentations are chemical events and must have chemical causes.
>
> quoted by HOPKINS (1913), p. 154

Finally Traube already in 1858 recognized that

> Ferments... are chemical substances similar to proteins that ... undoubtedly possess, just as all other substances, a definite chemical composition

and that they are

> not, as assumed by Liebig, substances in the process of decomposition that can convey their chemical movement to otherwise passive substances... Chemistry may... be able to explain physiological processes, but physiology cannot explain chemical processes... My more recent experiments have directly contradicted Pasteur's assertion that the alcoholic fermentation of sugars is tied to the respiration of yeast.
>
> TRAUBE (1877), referring to TRAUBE (1858)
> FRIEDMANN (1981), pp. 147–148

"Relatively little attention was given to Traube's views" (Fruton, 1972, p. 293), perhaps because of the greater renown of Pasteur, who attacked him along with Berthelot. Traube was the son of a wine merchant who took over his father's business. He never had an academic appointment. He was highly innovative in other fields as well. It was pointed out by Jacques Loeb (1906) for instance, that he was "the first to recognize that oxidations occur in the cells and not, as had been assumed before, in the lungs or the blood". He discovered the phenomenon of semipermeability, "probably one of the few major scientific discoveries made by a wine merchant in the attic of his store" (Friedmann, 1981).

In 1872 Marie Mikhailovna Manasseïn, based on 1871 work in the laboratory of Julius Wiesner in Vienna, published a report claiming alcohol formation in yeast extracts (Manasseïn, 1872). She concluded, in her words, that alcoholic fermentation was "not a physiological, but a mere chemical process". Liebig was aware of her work, and sent her an invitation to continue it in his laboratory. As she mentions in an 1897 publication,* written after reading about Buchner's work, she could not accept, because of family matters. In a blistering atttack on her work, Buchner and Rapp (1898) indicate that she was "subjectively convinced of the existence of an enzyme of fermentation, just as Traube and Berthelot had been before her", but that her experimental evidence was unconvincing. Thus she allowed dry heated or boiled yeast to stand for 2 to 56 days (!) with a sugar solution that could not have been sterile since it had been boiled for a mere 10 minutes. In other experiments she heated air-dried yeast for over 3 hours at 308 °C till it was charred, or boiled yeast for 45 minutes and claimed in all these cases to obtain fermentation. Buchner and Rapp graciously indicated that knowledge and methodology at the time did not permit her conclusions. Apparently her work does not appear to have elicited much, if indeed any, interest, although its ideas were, as recognized by Buchner and Rapp, ahead of their time. It is mentioned here because hers is the only report, before Buchner's work, of cell-free alcoholic fermentation.

In 1876 the word *enzyme* was invented by Wilhelm Friedrich Kühne (1876ab), professor of physiology at the University of Heidelberg, where in 1871 he had succeeded the great Hermann von Helmholtz. As pointed out by Gutfreund (1976), Kühne was given the name Willy, but changed it to Wilhelm Friedrich. Readers may draw their own conclusions. Kühne was a very versatile physiological chemist, and a master in the invention of new words. Thus he introduced the term *visual purple,* discovered and named myosin, discovered trypsin which he named in the same paper where the word *enzyme* was first

* See the chapter by Bohley and Fröhlich (pp. 56–57).

used. From the perspective of fermentation it is of interest that Berthelot's 1860 work on the inversion of cane sugar by a yeast extract played a prominent role in Kühne's preference for a new word in place of the cumbersome phrases "unformed" or "unorganized" ferments that had up to then been applied to the few soluble or extractable so-called ferments, in contrast to the unextractable and therefore "formed" or "organized" ferments. Kühne with his new word intended to simplify nomenclature and to codify the perceived qualitative difference between "unformed" and "organized" ferments. *Ferment* was to remain ferment, something inextricably related to life processes, and *enzyme* was invested with a more chemical aura of existence and function. One often forgets that our use of the word *enzyme* goes beyond the original intent and pays obeisance to a wrong theory and to a long-discarded view of vital processes (cf. Friedmann, 1981, p. 112). It is worth noting that the words *ferment* and *enzyme* are both related to yeast: *ferment* is an old term for yeast, derived very directly from the agitating nature of a fermenting sugar solution, and *enzyme* was to denote "in yeast", i.e. found in yeast, rather than being an intrinsic, life-bound part of it. Felix Hoppe-Seyler, the doyen of German physiological chemists, strongly attacked Kühne in the sarcastic tone that is so common in the scientific literature of the 19th century:

> Recently Kühne found it necessary to oppose my distinction [between ferment as chemical substance and ferment as organism which produces that chemical substance], but since he gives absolutely no reason worth noting in favour of his position I do not consider it necessary to say anything in reply. The new word, enzyme, can be added to the large number of new names that Kühne has invented, all of which denote substances that are still completely unknown.

HOPPE-SEYLER (1878), my translation

It is of some interest that new words tend frequently to be greeted with condescending rejection. Thus Berzelius's word *catalyst* had been scathingly rejected by Gerhardt:

> To call the phenomenon catalytic is not to explain it; it is nothing but the replacement of a common word [contact action] by a Greek word.

GERHARDT (1856), quoted and
translated by FRUTON (1972), p. 48

An amusing critique of the invention of new chemical words in found in Balzac's *La Peau de Chagrin* (1831), quoted and translated by Fruton, (1992, p. 235):

> "Well, my old friend,... how goes it in chemistry?"
> "It is asleep. Nothing new..."
> "If one is unable to produce new things... it seems that you are reduced to inventing new names."
> "That is indeed true, young man!"

Words have a habit of leading a life of their own. Although Buchner's work helped to abolish the conceptual distinction between the more chemical enzyme and the more vitalist ferment, and hastened the disappearance of the latter term, the word "enzyme" did not find immediate or universal acceptance. The eventual effect of the new name is well summarized by Kohler: "When the term 'enzyme' lost the precision of Kühne's definition, it acquired the scope of Liebig's 'ferment'" (Kohler, 1973, p. 193). It is in this light that one can understand Eduard Buchner's statement (1907, p. 119), so peculiar to our ears: "The difference between enzymes and micro-organisms is clearly revealed when the latter are represented as the producers of the former, which we must conceive as complicated but inanimate chemical substances." Some five years after Kühne and fifteen before Buchner the title of a monograph: *Die Lehre von den chemischen Fermenten oder Enzymologie* (Mayer, 1882) provides an interesting early hint of transition. This appears to be the first use of the word "Enzymologie". Apparently the word "Fermentologie" was never used, earlier or later. The emphasis is on "chemical ferments" in contrast to "organized ferments" that are considered less or not at all "chemical" (Meyer, 1879). The title of O'Sullivan and Tompson's classic study (1890) on the formation of enzyme-substrate complexes includes the phrase "enzyme or unorganized ferment". Emil Fischer used the word enzyme in his classical 1894 "lock and key" study. Jean Effront's 1899 textbook has the title *Les Enzymes et leurs Applications* (a title directly translated in the 1902 English version), although in this book the words *enzyme, ferment, diastase* are used interchangeably with wild abandon. A. J. Brown, shortly after Buchner (1902) used the term *enzyme* in his influential enzyme–substrate paper. This paper has in fact what is probably the shortest title for a scientific paper: it is simply called *Enzyme Action.*

There were some persistent holdouts. Arthur Harden and William John Young, in their classic studies on alcoholic fermentation, referred to the alcoholic ferment of yeast juice in titles to papers between 1904 and 1911 (see Harden 1932, pp. 214–215). Carl Oppenheimer's exhaustive scholarly treatise was called *Die Fermente und ihre Wirkungen* through many editions until the last supplement to be published (1939). One of the greatest enzymologists, Otto Warburg apparently never used the word *enzyme.* He resolutely held on to the term *Ferment* (1938, 1946), and in a classic book he still referred to the glycolytic enzymes as *Gährungs-fermente* (awkwardly translatable as "fermentative ferments"). By now the word enzyme has replaced all the former expressions, and at long last vitalist residues inherent in the word ferment have all but disappeared.

Between 1876 and 1879 Louis Pasteur and the eminent chemist Marcellin Berthelot engaged in a verbal duel of recrimination on their differing views of fermentation. This celebrated *querelle des fermentations* (Pasteur, 1876, 1878abcd, 1879abc; Berthelot 1876, 1878, 1879abc) is in fact a detailed examination of the ideas behind the distinction between formed and unformed ferments that led to the earlier formulation of the word *enzyme,* although these terms are not found here. Berthelot, near the end of his first contribution to the debate (Berthelot 1876) states that one must make a "distinction between the chemical role of microscopic beings which secrete ferments and that of the ferments themselves", while Pasteur begins his reply to this statement with the equally clear and apparently not very different statement: "In fermentation proper one has to consider two essential things: the agent that brings about the fermentation and the mechanism of action of this agent." Yet their viewpoints are very different indeed, Berthelot taking the position of the chemist, and Pasteur that of the biologist. Berthelot (1878) asks the question:

> It is a matter of knowing if the chemical change, brought about in all fermentations, cannot be resolved in terms of a fundamental reaction, brought about by a defined special principle, of the class of soluble ferments… One has to know how to isolate it, i.e. to ascertain the special conditions under which the soluble ferment is secreted in larger amounts than those under which it is consumed.

and Pasteur counters:

> By what subtle dialectical trick [*artifice*] can Monsieur Berthelot produce assertions… so contrary to the evidence?… Our colleague is the author of three hypotheses concerning the possible existence of a soluble alcoholic ferment in

> alcoholic fermentations proper, namely: (1) In alcoholic fermentation a soluble alcoholic ferment is perhaps formed. (2) This soluble ferment is perhaps used up in proportion to its production. (3) Perhaps conditions exist under which this hypothetical ferment would be produced in larger amounts than the quantities that are destroyed. These hypotheses of Monsieur Berthelot are absolutely gratuitous; never, as far as I know, has our colleague taken the trouble of presenting them honourably to the public, i.e. by accompanying them with personal observations and experiments.
>
> PASTEUR (1878d)

At the end of this communication Pasteur is even more strident:

> It is possible [for some one] to agree with me when, first, it is accepted that fermentations proper require as an absolute prerequisite the presence of microorganisms… Will Monsieur Berthelot or will he not contradict [this position]… not with *a priori* points of view, but with serious facts? If yes, let our fellow member [i.e. of the Academy, doubtlessly intended sarcastically] have the kindness to say so; if no, there is nothing for us to discuss.

This salvo occurred halfway through the debate: it did not stop here! Pasteur, in the last of his eight communications (Pasteur 1879c) eloquently discards the notion of a relation between fermentation and catalysis and takes on the names of the cream of chemists of his time and shortly before:

> Berzelius, Mitscherlich, Liebig, Gerhardt, Monsieur Frémy, Monsieur Berthelot and many other observers ascribed the probable cause of fermentative decomposition to a *catalytic* [Pasteur's italics] presence, to use the word of Berzelius, or to a movement imparted by dead matter in the process of alteration. In a word, the mystery was so great that one had to resort as an explanation to downright occult forces.

Nowhere does Pasteur mention directly that he himself had tried very hard indeed to obtain yeast extracts that could ferment sugar. There is no doubt, however, that he carried out such experiments, and that he did so to the point of not taking holidays and working himself to near exhaustion. In a passage whose tone is perhaps unique in the scientific literature (Pasteur 1879a), we read:

> Never, perhaps, in my already long career, have I worked so hard [*je n'avais fait tant d'efforts*] as during the year 1878…; never, subsequently, did I have such an overpowering [*impérieux*] need for rest. Well, I devoted all my recent holidays to the experimental checking of the posthumous writing of Bernard, and as a result I still feel extremely tired. I did what Monsieur Berthelot should have done before publishing the Notes of our dear and missed colleague.

After self-pity a somewhat ill-tempered nudge at his antagonist! First, the reason for Pasteur's intense preoccupation with the nature of alcoholic fermentation: as we saw, Berthelot had shown in 1860, i.e. almost 20 years earlier, that a ferment could be extracted from yeast, and that he concluded the living being not to be the ferment, but to give rise to it. Here there was no mention of fermentation proper. The reason for Pasteur's interest with the nature of alcoholic fermentation and for much of the Pasteur–Berthelot debate, was that the "illustrious physiologist" (Pasteur, 1878c) Claude Bernard upon his death in February 1878 had left behind a hidden manuscript, written October 1877, which was discovered in Bernard's country house by one of his young assistants, Jacques Arsène d'Arsonval.

This manuscript was published, according to Pasteur with mistakes (Pasteur, 1878c), in the *Revue Scientifique* at the instance of Berthelot. A theory of alcoholic fermentation was proposed that included the notion that alcohol is formed by a soluble ferment in maturing or rotten fruit. These ideas of the physiologist Bernard were, of course, very much in accord with those of the chemist Berthelot, and this is almost certainly the reason that Berthelot saw to it, to the dismay of Pasteur, that this preliminary manuscript was published. It is not clear, as discussed by Pasteur (1878c), whether this manuscript represents experimental results or only a theory. In a remarkable exhibition of pique, Pasteur, on learning of this manuscript, said:

> My surprise grew when I realized that all these Notes were written by Claude Bernard from the 1st to the 20th of last October [1877] in his country house of Saint-Julien, near Villefranche, that Claude Bernard spent the months of November and the month of December among us, attending our meetings in good health, seated to my right, as you know. Now, he told me not a word about his new experiments. Is it not strange that he, so frank, so open, so prone to free discussion, who did not cease to show me the most

> friendly affection, who, every week… spoke with me, at this very spot about fermentation, should on his return to Saint-Julien have possessed convincing proof that I was completely wrong, and that he would have hidden this from me without even the slightest allusion? It appears to me that this does not seem possible.
>
> PASTEUR (1878a)

Pasteur knew, of course, that yeast is absent from sour grapes, and that it appears on ripening grapes, a point that he made in his *Studies in Beer* and to which he refers in the present debate (Pasteur, 1878c). This is undoubtedly his rationale for using yeast to test the truth of Bernard's writing. It is important to understand the reasons for Bernard's interest in the field, since these directly contradicted Pasteur's views on fermentation, to Pasteur's annoyance. We saw above that Bernard, in a partial retreat from vitalist thinking but in a reaffirmation of vitalist ideas in regions where these could not be disproved, strongly distinguished between degradative, chemical events which he viewed as allied to death, and synthetic events, allied to life. In Bernard's opinion alcoholic fermentation was a degradative act, and it should therefore be possible to reproduce it chemically in yeast extracts in analogy to other degradative activities such as Berthelot's inversion of cane sugar.

This view of the significance of alcoholic fermentation was diametrically opposed to Pasteur's view that held fermentation to be related to the life, and therefore by implication not to the death, of the yeast cell. Pasteur states at the end of one of his notes, July 29, 1878 (Pasteur, 1878b):

> I am always determined to repeat Claude Bernard's experiments… I have decided to do this on a scale and with an abundance of data that the subject deserves [*avec une ampleur de résultats dignes du sujet*], and out of the respect that we owe to the memory of our colleague whom we miss.
>
> My translation

Pasteur (1876) had stated, even before he knew of Bernard's manuscript "As to the mechanism of fermentation in general, I look for it without any preconceived ideas".

There is excellent independent confirmation that Pasteur was in the latter half of 1878 busy with attempts to prepare active yeast extracts. We have it from an 1898 lecture, i.e. a year after Buchner's discovery, by Émile Roux, Pasteur's long-time associate, that

> Pasteur himself carried out experiments on this subject. I remember that, at the time that I joined his laboratory, he tried to extract the alcoholic ferment from yeast cells by grinding them in a mortar, by freezing them to make them burst, or by putting them in concentrated saline solutions in order to force the sap to leave through the cell wall by osmosis. All in vain. Pasteur did not find alcoholase, so that if he thought its existence to be possible, he did not think that it really existed [*si bien que s'il croyait son existence possible il ne pensait pas qu'elle fût une réalité*].
>
> ROUX (1898, my translation)

It turns out that Roux was accepted as an assistant in Pasteur's laboratory in November 1878 (Delaunay 1975), so the timing of the Pasteur–Berthelot debate and of Pasteur's own complaint about his hard work on fermentation in 1878 are in perfect agreement. There is excellent direct evidence as to why Pasteur failed: it was not that his methods were at fault, but rather that the yeast that he employed was unsuitable. We will discuss this after discussing Buchner's success with yeast extracts.

Pasteur (1879c) had the final word in this lengthy exchange with a self-boosting passage on "the rigorous judgment of a scientist in terms of the conclusions that he draws from his experiments", written about him as far back as 1861 by Michel Eugène Chevreul. At the end of their debate Berthelot and Pasteur had not changed their positions. The former held that the inability to obtain sugar-fermenting extracts from yeast was due to an inherent instability or self-consumption of the chemical constituents of the yeast in the act of extraction or assay, while Pasteur held to his earlier views on the indissoluble correlation between fermentation and the life of the yeast cell. "These two different interpretations of similar data are an object lesson in the facility by which the lure of negative results can feed the complacency of prior conviction" (Friedmann, 1981). In the words of Pasteur's successor as director of the Paris Pasteur Institute, and his first biographer, Pierre Émile Duclaux (1896, p. 266), in what has been called "one of the most impressive and perceptive books ever written on the development of a scientist's thought" (Geison, 1974):

> Pasteur came out of [this discussion] more fixed in his ideas, and Berthelot, apparently without having yielded any of his.

> This should lead as to distrust all discussions, even scientific ones.
>
> DUCLAUX (1896, p. 266)

This debate is probably unparalleled in the scientific literature in its length (about 60 printed pages), its vacuousness (no new facts are ever given), its intensity, its repetitiveness, its exquisite elegance (the beauty of style makes for engrossing reading), its tenaciousness and vitality (there are no fewer than six communications between January 6 and the last communication, on February 10, 1879) and its high drama (one follows the discourse of the two scientific giants with bated breath, for one simply does not know with what new skill charges are refuted and countercharges mounted). The death of Claude Bernard and the ambiguity of his hidden manuscript add to the mystery-story flavour of the debate. The polemics of Pasteur and Berthelot were strictly professional and not personal. Thus Berthelot wrote a very friendly letter to Pasteur in 1879 on the occasion of the marriage of Pasteur's daughter, and Pasteur replied in the most cordial terms: "The scientific discussions in which I have engaged have never suggested the least bitterness towards my adversaries" (see Velluz, 1964; "Chemicus", 1974). The debate is very much in the spirit of one of the many drawings of Honoré de Daumier (who died in 1878, the year of the apogee of the Pasteur–Berthelot dispute): two lawyers hug each other after forcefully arguing on the opposite sides of a given case.

With the close of the Pasteur–Berthelot debate a hiatus of neglect falls on the field of the exploration of alcoholic fermentation. As we learn from Arthur Harden's superb historical introduction to his book, *Alcoholic Fermentation,* there were some more unsuccessful attempts at extracting active extracts from yeast (Nägeli and Loew, 1878, Mayer, 1879), and one more theory of alcoholic fermentation (Nägeli, 1879), a "somewhat complex" one that combined features of various earlier theories and that "lost all significance" (Harden 1932, pp. 15–16). The field now lay as it were dormant for nearly twenty years "and then in 1897 the question which had aroused so much discussion and conjecture, and had given rise to so much experimental work, was finally answered by Eduard Buchner" (Harden, 1932, p. 16).

It is usually stated that the actual observation by Eduard Buchner of fermentation in a yeast extract was accidental. The extract was prepared in his brother Hans's laboratory in Munich by a procedure worked out there by his assistant, Dr. Martin Hahn (sand-grinding, followed by admixture of kieselguhr and application of hydraulic pressure). The qualification of accidental is correct insofar as various other preservatives, after unsuccessful trials with various antiseptics, were tried to prevent coagulation on standing, among them a 40 per cent solution of sucrose. The sucrose — hence the accident — not only worked as preservative, but turned out to become decomposed, as shown by the tell-tale bubbles that Eduard Buchner, just in for his vacation from the University of Tübingen, observed while Hans and Martin were just out of Munich for their vacation. Musical chairs, vacation spent in a laboratory, preservative possibly added in part because of the dictates of vacation time, quite a story of a coalescence of improbabilities.

There is rather more to the story, however, usually not quite as well recognized. First, Hans Buchner, a well-established immunologist, who in addition happens to be the inventor of the method for the growth of anaerobic bacteria under a pyrogallol seal, a method prevalent before the much later introduction of the Hungate anaerobic techniques, was interested in preparing bacterial extracts for the study of his so-called allexines, which have been completely forgotten in the meantime. The complex story, with a strong emphasis on the immunological theory, has been told in a masterly fashion by Kohler (1971, p. 52):

> It was about... 1893 that the first experiments were done by Hans and Eduard Buchner on breaking open yeast cells by mechanical grinding, and there can be little doubt that the motive behind these experiments was Hans's hope of revolutionizing the theory and practice of serum therapy.

It is of interest, as Kohler stresses, that by 1897 there had been a "spate of sand-grinding" of various microorganisms by a large number of workers, including Emil Fischer, and that Eduard Buchner actually applied in 1893 for a sand-grinding technique patent that was rejected, presumably because, as Kohler says, his application for a patent "began to look somewhat presumptuous" in view of the prevalent successful use of this technique. Buchner was interested in opening up microorganisms to help his brother, and not out of an interest in obtaining cell-free fermentation. From this viewpoint he was really going to work on the preparation of bacterial extracts, but he used yeast since "in a brewing town yeast was cheap and readily available" (Kohler, 1971, p. 55). Although extracts could indeed be prepared from sand-ground yeast, the isolation of the soluble proteins from the sand and cell debris "proved unexpectedly difficult" because of the "repeated dilution with water and simple filtration... [which led] to loss of material and loss of activity due to dilution" (Kohler, 1971, p. 56). Eduard Buchner left Munich in 1893 for a university position, and the grinding experiments were stopped. Hans Buchner "pursued his studies of alexines, and Eduard continued… more pedestrian chemical researches" (Kohler, 1971, p. 58). Hans Buchner's interest in the preparation of bacterial protein never lagged. In 1894 he was appointed to the chair of hygiene and to the directorship of the Plant Physiological Institute in Munich. In the summer of 1896 he returned to the problem of preparing bacterial protein, and he gave this project to Martin Hahn, who solved the problem "in most ingenious way" (Kohler, 1971, p. 59), since, contrary to the earlier extraction methods by dilution that Eduard had tried, the use of a hydraulic press after grinding, addition of kieselguhr and wrapping in a cloth provided *undiluted* intracellular juice. This was the trick, and the rest, as one says, is history.

A few more points have to be made. Many workers, for example Amthor (1892) and Emil Fischer (1894), had used glass powder and not sand to grind up their microorganisms. Buchner in his very first paper on alcoholic fermentation (1897a) as well as in his part of the book *Die Zymasegärung* that he wrote with his brother and with Martin Hahn (1903, p. 58) emphasizes the importance of not using glass powder because of its weak alkalinity. It must be remembered that biological extractions for many years continued to use water; the invention and use of buffers came a few years later (e.g. Fernbach and Hubert, 1900; Michaelis and Davidsohn, 1911; and especially Sørensen, 1909) The importance of avoiding alkalinity in the Buchner–Hahn yeast extract preparation is also pointed out in a masterly recent article by Lothar Jaenicke (1997).

An intriguing question must be asked: Why did Pasteur, Berthelot and others (see Kohler 1971, pp. 39–40; Fruton, 1972, pp. 62–63) fail to prepare active yeast extracts? Since Pasteur did not publish his negative results we do not know any details of his methods, except that, as Roux (1898) pointed out (see above) he tried many different approaches. It was the Nobel laureate Arthur Harden, the first after Buchner materially to advance our knowledge of Buchner's zymase (requirement for phosphate, participation of a dialysable material, now called NAD, originally named co-zymase) who did the critical experiment. He found Paris yeast not to work:

> The nature of the yeast is of particular importance. Thus while Munich (bottom) yeast usually gives a good result, a top yeast from a Paris brewery was found to yield extracts

> containing neither zymase nor its co-enzyme in whatever way the preparation was conducted. The existence of such yeasts is of great interest, and it was probably due to the unfortunate selection of such a yeast for his experiments that Pasteur was unable to prepare active fermenting extracts.
>
> HARDEN (1932, p. 25)

Buchner himself, in the second of his series of publications on cell-free alcoholic fermentation, had noted marked differences between different yeasts in yielding active cell-free extracts (Buchner 1897b). (For a very recent and scholarly book on the impressive variety of yeasts, see Spencer and Spencer, 1997).

> The circumstance that, as we now know, yeast happens to have a notoriously tough cell wall, and the accident that Pasteur and Berthelot used a strain of yeast that gave negative results, enabled the various promulgators of the biological and of the chemical viewpoints to content for supremacy for many years. Our story shows that the development of science is more than the recitation of an apparently inevitable uni-directional dictatorship of facts over the prejudice of fashion. A powerful additional determinant of this development is provided by the interplay of personalities and by the manner in which personalities weave fashions into the selection and interpretations of their facts.
>
> FRIEDMANN (1981, p. 672)

A reading of Buchner's first paper on cell-free alcoholic fermentation, reproduced in English on pp. 25–31 of the present book, reveals decided and fascinating residues of vitalist thinking, a strong reminder of the victory of custom over the dictates of observation. Thus one finds an interesting confusion between the view (p. 27) of zymase, the sugar-fermenting enzyme, as "a dissolved substance, undoubtedly a protein", and the view (p. 28) of zymase "as a genuine protein... much closer than invertin [Berthelot's enzyme] to the living protoplasm of yeast cells." More interesting is the acceptance by Buchner of a fundamental difference between zymase and "the established list of enzymes" (p. 27), all of which "merely bring about hydrolyses that can be imitated by the simplest chemical means" (p. 28), an idea fortified or suggested by the inability, in contrast to observations with invertin, to maintain zymase activity upon precipitation with alcohol (p. 28). Mention is made of Baeyer's ideas of alcoholic fermentation as a chemical process, but an air of mystery is allowed to remain about the possible chemistry of the fermenting process, perhaps unexpected from a person with a rigorous training in chemistry. In spite of Buchner's realization that his results overthrew Pasteur's dominant view on fermentation ["We all grew up in the atmosphere of Pasteur's views… I hence understandably was very sceptical when I… obtained experimental facts that appeared to indicate cell-free fermentation" (Buchner, 1898)],* it is clear that residues of the attitude that informed this view remained, but not for long: in his Nobel Lecture, Buchner discusses at length various chemical means, studied in several laboratories, to convert glucose to carbon dioxide and alcohol (Buchner, 1907, p. 116). In fact, Buchner was closer to the actual process of glycolysis than is generally realized. It is well known that Aleksandr Nikolaevich Lebedev's method of using air-dried yeast as a ready source of zymase displaced Buchner's method (Shamin, 1990, cf. Harden, 1932, pp. 24–26). What is much less well known is that Lebedev worked in Buchner's laboratory in 1907, and there began "his lengthy research on the chemical nature of alcohol fermentation", leading in 1909 to the "first scheme of alcohol fermentation" (Shamin, 1990), with the main role played by glyceraldehyde and dihydroxyacetone, and leading three years later to the corresponding phosphates. Thus this work provided a direct connection to the later work of Gustav Embden and of Otto Meyerhof. Although Lebedev had left Buchner's laboratory by 1909, there is no doubt that it is there that the seeds for his work were planted.

Buchner's results were attacked from various sides, but within a few years they were almost universally accepted (see Kohler, 1972; Fruton, 1972, p. 86). A highly amusing rejection was by a certain J. Reynolds Green, ScD, FRS, who already in 1897 dismissed Buchner's work: "For the present... I must contend, in opposition to Buchner, that at any rate our English Yeasts do not contain any alcohol-producing enzyme" (Green, 1897). He later became a strong defender of Buchner's views (Green, 1898). Another powerful acceptance came from Max Delbrück, the influential head of the Institute for Brewery Research and Teaching (Versuchs- und Lehranstalt für Brauerei) in Berlin (uncle of the future Nobel Laureate), after an initial rejection. He calls zymase "the enzyme par excellence, justly called zymase", a sentiment not perhaps surprising from someone whose interests were

* This lecture, on 14 March 1898 before the German Chemical Society in Berlin, is quoted at greater length in the chapter by Bohley and Fröhlich (pp. 54–55).

centred in brewing. It is most interesting that the first public acceptance of Buchner's work came already in 1897 from none other than Pierre Émile Duclaux, Pasteur's first biographer, his immediate successor as director of the Pasteur Institute in Paris, and founder as well as editor of the influential *Annales de l'Institut Pasteur*. One would have expected the new findings to be opposed by a disciple of Pasteur, since they could be readily interpreted to conflict with the views of the recently deceased (1895) Pasteur. However, Duclaux's respect for experimental observation, similar to Pasteur's, prevailed, and he did not feel that Buchner's findings contradicted Pasteur's position. He wrote in the *Annales* in one of several 1897 papers championing Buchner's work (see Kohler 1972, p. 338):

> Some scientists fully accept this discovery and even derive extreme conclusions from it by pretending that it overturns Pasteur's doctrine on fermentations. Pasteur's doctrine will be overturned the day that alcoholic fermentation will be achieved purely chemically and without any vital activity. But as long as yeast is needed to produce alcoholic diastase, Pasteur's theory can express what the master himself would have said: Here is yet another vital activity [*action vitale*] which is manifested by a chemical mechanism.
>
> DUCLAUX (1897, p. 348, my translation)

This position resembles that taken already in 1858 by Moritz Traube (Traube 1858). A year later another former close Pasteur associate, Émile Roux, made a statement similar to Duclaux's but the emphasis is rather more chemical:

> Certainly, the decomposition of sugar by alcoholase is a purely chemical reaction, but the formation of the enzyme is an act associated with life, and since it is not yet possible to make alcoholase without a living cell it follows that alcoholic fermentation remains correlated with the life of the yeast.
>
> ROUX (1898, p. 839, my translation)

Buchner's own position vis-à-vis Pasteur was very modest:

> The famous Frenchman's theory consists of a physiological part: fermentation is life without oxygen, that is, fermentative organisms obtain their reservoir of energy by the process of

> fermentation, while the other living beings obtain it by means of respiration; and the theory consists of a fermentative-chemical half: no fermentation without organisms. The first statement is not at all changed by the discovery of zymase; the second statement requires only one modification: no fermentation without zymase, which his formed in organisms.
>
> BUCHNER and RAPP (1898, p. 211, my translation).

Perhaps Bernal (1954, ch. 9, sect. 5) put it most succinctly:

> In the end both von Liebig and Pasteur were right. Fermentation is brought about by a ferment, but that ferment can only be elaborated by a living organism.

However it is important to stress that the view of Duclaux and Roux were not shared by all scientists. Many still argued that yeast extract contained "living protein" or "bits of protoplasm" (see Fruton, 1972, p. 86). Kohler makes the important point that "Buchner's 'proof' of the chemical view did not seem so unambiguous at first. Biologists' reactions to zymase correlate very closely with their previous disciplinary commitments" (Kohler, 1975, p. 295). "Initially, at least, zymase was less a determinant of opinion than a touchstone of pre-existing opinion… The primary effect of the zymase debate… was on those who were already inclined to the new view" (Kohler, 1972, pp. 351–352). In the long run it was time, not argument, that prevailed over fashion. "Conceptual change came about not by wholesale conversions of individuals but by a process akin to natural selection, whereby the composite character of a population changes" (Kohler, 1975, p. 295). The zymase debate occupied Buchner until his last papers.

We have here and there indicated that the sway of vitalist ideas did not meet universal acceptance in the 19th century. We mentioned Georg Sigwart, Marcellin Berthelot and Moritz Traube, Felix Hoppe-Seyler. One has to bear in mind that the narrow specialities with which we classify modern scientists did not hold sway in the 19th century, but it is of interest that groups of scientists whose primary concerns were not specifically chemical also tended to reject vitalist attitudes in favour of chemical or physicochemical ones. Thus already around 1847 "a quadrumvirate of rising physiologists" — Emil du Bois-Reymond, Ernst Brücke, Hermann von Helmholtz, and Karl Ludwig — decided to reject "any explanation of life which appealed to nonphysical vital properties or forces" (Turner, 1972). In the words of du Bois-Reymond, "Brücke and I have sworn to each other to validate the basic truth that in an organism no other forces have any effect than the common physicochemical ones..." (quoted by Lesky, 1973). The physiologist du Bois-Reymond vigorously opposed the introduction of physiological explanations in terms of a vital force. That force provided no explanation and was

> but a comfortable resting place where... reason finds peace in the cushion of obscure qualities... If one observes the development of our science he cannot fail to note how the vital force daily shrinks to a more confirmed realm of phenomena, how new areas are increasingly brought under the dominion of physical and chemical forces... It cannot fail that physiology, giving up her special interests, will one day be absorbed into the great unity of the physical sciences; [physiology] will in fact dissolve into organic physics and chemistry"
>
> quoted by TRUSTED (1996, pp. 118, 151).

An interesting case is that of Theodor Schwann. While Eduard Buchner in his book has a quotation (p. 48) from Max Delbrück (1898) that indicates Schwann to have been a vitalist, a revealing passage by Schwann (1878) à propos the vital force is quoted by Marcel Florkin (1975):

> A simple force different from matter, as it is supposed, the vital force would form the organism in the same way as an architect constructs a building according to a plan, but a plan of which he is not conscious. Furthermore, it would give to all our tissues that which is called their proper energy, that is, the properties that distinguish living tissues from dead tissues: muscles would owe it their contractility, nerves their irritability, glands their secretory function. Here, in a word, is the doctrine of the vitalist school... I have... always rejected as illusory the explanation of vital phenomena as conceived by the Vitalist school. I laid down as a principle that these phenomena must be explained in the same way as those of inert nature.

Rudolf Virchow, "the most prominent German physician of the 19th century" (Risse, 1976), was very concerned with questions of vitalism. He wrote a book with the title *Alter und Neuer Vitalismus* ("Old and

New Vitalism") (Virchow, 1856) and is credited with the promulgation of a so-called neovitalism. In 1849 he decided to accept a special vital principle whose centre was the living cell. On the other hand, almost fifty years later, in a lengthy lecture given (in English) at the age of 77 in London on the occasion of the opening of the Charing Cross Hospital Medical School, we find the following:

> How can a single power, whether we call it in the spiritualistic sense spirit, soul, *spiritus rector,* or, in the physical sense, vital force or electricity, build up… diverse organisms?… Cells are composed of organic chemical substances, which are not themselves alive, but the mechanical arrangement of which determines the direction and power of their activity.
>
> VIRCHOW (1898)

On the other hand, one here also finds the following statement, which could have been written by Claude Bernard forty years earlier:

> These two kinds of substances, the living and the non-living, cannot be identified with one another. In spite of chemical similarity or even correspondence, they exhibit recognizable differences, not alone physiological, but also mechanical and physical.

In the words of Diepgen (1952, quoted in Selberg and Hamm, 1993; my translation):

> Virchow attacked the difficult problem [of vitalism] with the whole universality of his scientific and philosophical approach. All of cosmology, geology and paleontology, Newtonian physics, chemistry, especially ferment chemistry and catalytic phenomena, and especially the new observations in biology were included in the range of his proofs. On this basis the force that acts in the organism, and especially in the cell becomes for him a mechanistic and uniform vital force which is bound to matter.

Posner (1921) discusses Virchow's attempts to reconcile his early purely mechanistic, i.e. physical or chemical, view of vital phenomena with his later ideas on a special vital force, and concludes: "It is scarcely possible to say that he penetrates [this matter] with complete clarity" (my translation). The correspondence, extending over a period of thirty years (1864–1894), between Virchow and du Bois-Reymond which among many topics touches the above, has recently been published with an illuminating editorial introduction (Wenig, 1995). Virchow's fluctuations between acceptance and rejection of a vital principle can be regarded as symptomatic or representative of the 19th century fascination with this topic, its divisiveness and its uncertainty.

Paradoxically, it was precisely in the area of biological research closest to chemistry, areas that we would now call biochemical, as exemplified by the Pasteur–Berthelot altercation, that the vitalist, non-vitalist debate held centre stage, and it is therefore perhaps not too surprising that it is precisely here that a decisive observation had by far the greatest impact. So, from the viewpoint of Buchner's work, his experiment struck home, as it were, far more strongly than the generally proclaimed views of principles and attitudes that one way or the other failed to convince the majority, because of a mixture of attitude and the lack of decisive observations.

The experimental results that follow from the physicochemical approach to living processes are due not only to properties intrinsic to the phenomena selected for study but also to the operation of the selection process itself. The physicochemical approach has been singularly successful because, in Victor Henri's words (1903), it can "be analysed experimentally and hence can permit a deeper understanding of the mechanism of the phenomena studied." This deeper understanding is made possible not only in terms of the physicochemical results by themselves but in terms of their wider implications. The physicochemical approach, which has been so productive in the inanimate world, has now become the stock-in-trade not only of the biochemist's but also of the biologist's study of living systems. This approach continues to encroach on domains of biological thinking from which it had originally been deliberately excluded.

The physicochemical approach is not at all new in Western thinking. At the very beginning of Greek philosophy, we find Thales' notion that one substance, water, is the substratum of nature. In contrast to such analytical ideas, we find the idea of a vital principle such as Aristotle's entelechy. Thus the "sharp division… between those who stressed the uniqueness of living matter and those who believed the body to be a mechanical engine" (Leicester, 1974, p. 111) has a venerable tradition. It is possible that nonvitalist iatrochemical ideas could be formulated in the seventeenth century with somewhat greater ease than we might at first expect since they could be fitted into a framework of still prevalent alchemical attitudes. In later centuries, however, we see the development of a new attitude. There was no pervasive pattern of authority for the physicochemical view of living processes to fall back on; in order to be accepted, this view depended on the generalizing potential of "irreducible and stubborn facts" (William James, quoted by A. N. Whitehead, 1925) rather than on a pre-existing framework of assumed but unproven ideas.

It is often assumed that the postulate of a vital force stood in the way of an experimental approach to the study of vital processes: destruction of life, it might be held, entailed destruction of living events, which therefore were not amenable to experimental investigation. Historical facts do not, however, bear out such a simple cause and effect relation between ideas and experiments: the vital force was a gray eminence or convenient refuge that imposed limits on the scope of observation, but it did not stifle experimentation beyond those limits. Thus it would be truer to reverse the postulate: the difficulty in getting certain experimental results were consistent with, or suggested the existence of a vital force. As an example, Leopold Gmelin in the *Handbook of Theoretical Chemistry* (1829), referring to plants and animals stated that what he called "Chemical Physiology" investigates the "chemical changes which occur in these bodies in so far as [*solange*] they are under the control [*Botmäßigkeit*] of the vital force" (quoted by Mani, 1956). Some thirty-odd years later, as we saw above (p. 81), Claude Bernard (1857) after his epochal isolation of glycogen from dog liver, strongly contrasted the basis of the "entirely vital" formation and of the "entirely chemical" breakdown of glycogen in the liver.

Jennifer Trusted (1996, p. 149) goes further. In a discussion of 19th century vitalism she states that

> appeal to vital forces was not intended to end further inquiry but to stimulate it; it was intended to encourage laboratory experiments designed to discover their mode of action. In this respect 19th-century vitalism, though couched in the language of the nature-philosophers (Coleman, 1977, p. 150), was very different from theirs... The later, 19th-century biologists and chemists saw experimental investigation as the basis for inquiry; they did not think the riddles of existence could be solved by thought (Nordenskiöld, 1928, p. 370).

Generalizations do not necessarily apply to all fields. It is likely that in our area attitudes did inhibit discovery. Vitalist attitudes existed here, as elsewhere, as a movable curtain of mystery that receded with new knowledge. Physico-chemical convictions would undoubtedly have hastened the search for cell-free fermentation. Thus Pasteur, driven by Berthelot's insistence that fermentation is a chemical process that does not need the living yeast cell, did look for fermentative activity in yeast extracts, and used his failure to support his vitalist convictions. Without Liebig's and Berzelius's unwillingness to accept yeast as alive, progress in the field would almost certainly have proceeded faster. On the other hand, failure in preparing active yeast extracts was not dictated by vitalist dogma but by experimental difficulties. Thus in the course of the 19th century, ideas promulgated without compelling evidence by some, such as Moritz Traube, remained little more than curiosities, and observations on soluble or so-called unformed ferments were regarded as insignificant exceptions to general rules. Only a compelling experiment could resolve the impasse. It is agreed (Kohler, 1971; Fruton, 1972) that the field of yeast fermentation lay quiescent for 20 years — starting with the end of the Pasteur–Liebig dispute (Liebig had died in 1873) — and was reawakened not by theory but by experiment, Buchner's experiment. This simple historical fact is a telling tribute to the persuasive power of observation over attitude. Buchner's accidental discovery of cell-free fermentation was not suggested by the presence or the lack of vitalist assumptions; it was simply an experimental result that dictated its consequences upon believers, and that required further experiments to help convert the unbelievers. As we saw above, Buchner himself found it hard to believe his own observations, because he, as so many others, were under the powerful sway of Pasteur's ideas. There is a slight possibility — although this may not be fair to Buchner as chemist — that in this case, as one of many, a residual vitalist abdication to rational inquiry was behind a certain lack of probing chemical questions. [Old ideas die hard. In the sixth (!) enlarged and revised English edition (1960) of Fritz Feigl's standard treatise *Spot Tests in Organic Analysis,* zymase is included (p. 633) in a list of "Individual Compounds" for which an identifying test is given.] On the other hand one might argue just as convincingly that lack of progress here was simply the result of the impossibility of predicting the "right" experiments. Here a good scientific "nose" or intuition carries far more weight than any philosophical convictions. It is fascinating to read Arthur Harden's lucid description of his and William Young's painstaking step by step work that led to the discovery of the role of phosphate and of co-zymase, a co-ferment or coenzyme, in alcoholic fermentation (Harden, 1932, pp. 42–75).

The impressive ability of chemistry to answer biological questions is often admired and often dismissed by the term *reduction* or *reductionist*. It must be emphasized that a fine line should be drawn between reductionist validity and existential identity. Reductionist attitudes fluctuate between two extremes: a belief on the one hand that the complex is, in fact, identical to the simple (in our case, that life is chemistry), and a belief on the other hand that for experimental purposes the complex has to be explained in terms of the simple (in our case, life in terms of chemistry). The former would say that reductionist validity is tantamount to existential identity, while the latter would only agree that reductionism is useful as an experimental device, that its application is derived from and limited to pragmatic validity. Reductionism in its extreme or existential form must necessarily dismiss the part as a representation of the whole, and here, therefore an entity that is not part of the whole takes the place of the whole. On the other hand, reductionism as a pragmatic device can very readily arrange aspects of the whole in a hierarchical order of perceived importance. Most would agree that the pragmatic validity of any selected aspect goes beyond descriptive or experimental convenience, that it in fact permits us to interpret and view the whole as a manifestation of the selected part: the part magnifies the whole and helps to reveal it as a new set of relationships; selection and analysis, are, perhaps paradoxically, a precondition for the discoveries of relationships that enrich the understanding of the whole. In terms of language, one tends to apply the term *reductionist* to theoretical, and the term *analytical* to practical concerns. A superb early example of the pragmatic validity of a chemical investigation of the cell is found in a short lecture *The Chemical Organization of the Cell* by Franz Hofmeister, in which he states that chemical analysis of different tissue constituents has provided a plethora of important findings, and that it turned put to have been a bit premature to assume that the destruction of the living cell completely destroys its vital functions. He stresses that it is only by such destruction that it was found possible to establish the presence in cell of agents, such as enzymes, that are active during life. This lecture, rewarding reading to this day, is an elegant vindication of the value of the analytical approach to the unraveling of vital processes, (Hofmeister, 1901). (For an appreciation of Hofmeister's work, see Fruton, 1992, Ch. 5.) Reductionism has often been rejected on first principles because of the predictive limitations of going from lower or simpler to higher or more complex, a problem that is enshrined by the word *emergence* and that has pretty much disappeared as a viable object of enquiry. The fascination with reductionism does not go away. A recent symposium *The Limits of Reductionism in Biology* (1997) has received probing reviews (Williams, 1997; Bray, 1997), aspects of which smack of at least a partial return to vitalist attitudes. The present writer has not yet seen this book, and so an independent evaluation is not possible, but the readers of the present volume will undoubtedly be delighted to know that the ancient debate on methods and conclusions in biology is far from over.

In the case of biology the immense and pervasive success of chemical approaches toward understanding, toward prediction and toward medical success has achieved, and continues to achieve, pragmatic validity. None of the various possible degrees of reductionism addresses itself to the ultimate meaning of reductionist validity, and it is in this search for significance (in our case, a search to answer the question as to the strength of chemistry to answer biological questions) that differences in attitude come to the fore. To cite a specific example, Fleming (1964) has made the important point that the decision by the physiologists du Bois-Reymond, Brücke, von Helmholtz and Ludwig to substitute physicochemical forces for vitalist ones "was intended as a program for research rather than the enunciation of a *Weltanschauung*... Mechanism was coextensive with scientific knowledge, but not with the range of legitimate curiosity." More than a hundred years later one can see a fascinating reversal: in the research laboratory one may on occasion find it useful to reject purely physicochemical approaches to one's experiments or questions, but it does not follow that one has to adopt a corresponding philosophical approach to nature. For example, K. F. Schaffner (1967) states:

> Given the current state of biological science, there may be good heuristic reasons for not attempting in all possible areas to develop physicochemical explanations of biological phenomena, and good reasons for attempting to formulate specifically biological theories. This, however, is an argument which supports an irreducibility thesis for methodological reasons. Any attempt to twist this into a claim of real irreducibility for all time is, in the light of recent work in molecular biology, logically untenable, empirically unwarranted, and heuristically useless.

The question of the relation between chemistry and biology is exactly analogous to the question in physics as to the relation between mathematics and the physical universe: Why does chemistry "work"?, why does mathematics "work"? Biology is unavoidably chemical, just as physics is unavoidably mathematical. It is fatuous to ask whether one could have predicted *a priori* that chemistry would be so immensely fruitful for an understanding and study of biology, and mathematics similarly essential for an understanding of physics. It should be clear that opposition to a chemical approach for the study of living phenomena, inherent in some vitalist attitudes and found in some modern analogies to vitalism, is tantamount to denying that mathematics is an essential prerequisite to an understanding of the physical universe. Physics evolved through a period when deductions based on mathematical analysis were rejected since they were incompatible with dogma, and biology will undoubtedly weather similar attacks, born of ignorance or prejudice, on the understanding of biological phenomena.

There is no doubt that the advances in enzymology in the last decade or so of the 19th century contributed massively toward a swaying of scientific attitudes away from vitalist approaches. However, the results obtained by enzymological and other studies did not suffice to limit the vagaries of biological thinking. Thus it was the embryologist-turned-philosopher Hans Driesch who at the beginning of the 20th century powerfully resuscitated vitalist ideas (Driesch, 1908) based, characteristically, on his own — and as it turns out, erroneously interpreted — important research results (see Fleming, 1964, pp. xxv-xxvi). In 1911 Jacques Loeb gave a celebrated address whose object was "to discuss the question whether our present knowledge gives us any hope that ultimately life, i.e. the sum of all life phenomena, can be unequivocally explained in physicochemical terms" (Loeb, 1912). These words, although advanced as a question, are strongly reminiscent of the "1847 School" of physiologists and of Berthelot's words in 1860. There is, however, a fundamental distinction: while earlier workers' views were dictated by faith based on induction, the latter's deductions more than fifty years later were fashioned by conviction based on further evidence.

Ernest Nagel stated in his classic book *The Structure of Science* (1961):

> Vitalism of the substantive type advocated by Driesch and other biologists during the preceding century and the earlier decades of the present one is now almost entirely a dead issue in the philosophy of biology. The issue has ceased to be focal, perhaps less as a consequence of the methodological and philosophical criticisms to which vitalism has been subjected, than because of the sterility of vitalism as a guide in

> biological research.

An earlier version of topics treated in this book was published (Nagel, 1950–1951) in which elegant and clear arguments are presented for the rejection of the organismic approach, which to a large extent has replaced the vitalist approach as an "alternative to physicochemical theories of living processes."

One cannot of course pin down a certain instant when a given idea formally disappears from the stage of accepted opinion. C. H. Waddington has described the process well: "around the time of my student days the whole controversy vanished" (Waddington, 1961). However, even later the vitalist attitude had not disappeared completely. We can cite two interesting examples. Richard Willstätter, Nobel Laureate in chemistry, prominent opponent of the idea that enzymes are proteins and of the use of column chromatography as an analytical method, stated in his very last paper (Willstätter and Rohdewald, 1940)

> It must be concluded that Buchner's press juice and macerated yeast react with sugar in a fashion which differs from that of living yeast.

He elaborated on this in his celebrated autobiography, published posthumously:

> Some fermentation potential can be isolated [from yeast], but I consider it different from the fermentation effect of the living yeast cell.
>
> WILLSTÄTTER (1949, p. 63, English translation 1965, p. 66)

Willstätter's views were not unique. Thus in 1940, again, F.F. Nord, who was to be the distinguished editor for thirty years (1941–1971) of the annual *Advances in Enzymology* stated in a detailed review on the mechanism of alcoholic fermentation:

> It is not conclusive if, from the enzymatic behavior of structurally destroyed systems… forceful conclusions as to the qualitative actions of the parent systems within the living cell are drawn.
>
> NORD (1940)

Francis Crick is quoted as having changed from physics to biology because "he was impatient to throw light into the remaining shadows of vitalist illusions" (Judson, 1979, 1996). In a series of lectures delivered in 1966 Crick examined various examples of vitalist writing from the preceding few years and concluded, reluctantly, that we are far from having seen the last of such ideas (Crick, 1966). Indeed, Michael Polanyi subsequent to these lectures forcefully argued for a vitalist type of approach to an understanding of biological phenomena (Polanyi, 1967, 1968). Jacques Monod (1970), again, finds vitalism not dead at all:

> Certain schools of thought… challenge the value of the analytical approach to systems as complex as living beings. According to the holist schools which, phoenix-like, spring up anew with every generation, only failure awaits attempts to reduce the properties of a very complex organization to the "sum" of the properties of its parts. A most foolish and wrong-headed quarrel it is [*C'est là une très mauvaise et très stupide querelle*].

In a highly perceptive article on the mechanism-vitalism controversy, Hilde Hein (1972) also concludes that this controversy is unending, but she refrains from taking sides. In her opinion the point of view taken by any one individual on this issue — and she quotes eminent contemporary biologists on both sides of the fence — is indeed determined not by the examination of scientific evidence, but by "attitudes and prejudices prior to inquiry." She contends that "the mechanism-vitalism dispute is but one of a number of… fundamental disagreements which will be perpetuated as a long as people ask questions and seek rational answers." It should be stressed that the notion of a vital force has had ramifications far beyond our rather confined biological focus. In the quotations from Bishop Berkeley and from William Cowper at the beginning of this chapter the connotations of the terms *vital flame* and *vital energy* include those that were assumed to hold for biology, but they are far broader. At the beginning of our century, the philosopher Henri Bergson's *élan vital,* the centrepiece of his theory of creative evolution, enjoyed a long vogue without any obvious profound effects on biological thinking.

With the rejection of a vitalist approach to a study of living phenomena, one does two things: one expresses a particular mental attitude, and one embarks on an experimentally feasible course of investigation. As a result of these two contributing factors, the phenomena assume a new, broad and, as pointed out, a self-consistent and cross-fertilizing meaning that could not have been predicted in every instance and in every detail. The physicochemical approach inevitably merges with the biological approach. A fresh and at times unexpected view of biological processes is obtained. The study of enzymes — an important exercise in this approach — has provided potent examples not just of the discovery of facts but of the accompanying change in attitudes, initiated by Buchner's work.

What was new with Buchner's discovery was not just the demonstration that a process previously considered indissoluble (word chosen deliberately) from living processes could be demonstrated to survive the intact organism and therefore be amenable to study of a kind impossible with the living cell (an insight that leads to the not always accurate but useful approximation *in vitro = in vivo*). What was new was the wider meaning of this relationship in terms of the implicit recognition that extract repeats or mirrors the living system, i.e. *extract repeats or mirrors process.* The notion of extraction for the preparation of natural products is not at all new. In this sense the preparation of enzyme extracts continues an old-established tradition. What was new with Buchner was the elevation of this time-honoured procedure to a demonstration that *tissue extracts could provide not just what have been called natural products, i.e. compounds synthesized in tissues, but in addition natural processes.* In the sense that extracted enzymes are necessarily also natural products — although never designated as such — their ability to survive the extraction process tells us not only something about their properties as chemical entities, but teaches us a far more important lesson, namely that we have here an illustration of the scientific principle, going back at least as far as Galileo, that abstraction from the whole is necessary for understanding of the whole. Enzyme extracts had, of course, been obtained earlier [according to Malcolm Dixon (1971, p. 16), as late as 1920 only about a dozen enzymes were known] and Buchner's active material, centred on the mystery of fermentation, had been looked for before. However, Buchner's discovery had a far greater impact on biological thinking and on the development of biochemistry than the discoveries of any of the previous enzymes because its basis had been the subject of a lengthy and acrimonious dispute between two scientific giants, Pasteur and Berthelot, with important views expressed by the likes of Traube and Hoppe-Seyler. Buchner's work was a lucky break not only because he happened, unlike Pasteur and others, to use a yeast that yielded active extracts, not only because he stumbled upon a discovery more or less by accident, but, far more important, because it so happens that the living cell can yield some of its most important secrets since destruction of the inner organization of the cell is not, as we now know, associated with destruction of the conformation of most of the proteins that constitute fundamental functional and structural components of the cell.

The equivalence between *in vivo* and *in vitro* aroused the opposition of the vitalistically inclined critics, and was accepted by the ever growing number of mechanistically inclined admirers. Interest in function predominated, at least initially, over interest in structure which was completely inaccessible and of very minor relevance. Classical biochemistry, the topic of metabolic maps that forms its core, developed without the detailed knowledge of protein structure that has become a major ingredient of what has come to be called structural biology. Biochemistry soon developed the use of homogenates (in the first 50 years of this century the biochemical literature was full of the word *Brei*), tissue slices, membrane fractions and components of organelles such as mitochondria, but these later studies were initiated with extracts, and such studies still continue. The *in vitro – in vivo* correlation, which refers not to compound but to activity, is now absolutely taken for granted as one of the paradigms or principles of the field. Any apparent exceptions are explained not, as they would have been tempted to be a hundred years ago, as a consequence of interference with a vital force, but rather to interference with *in vivo* controls at different organizational levels such as membrane structure, the interplay between organelles, the function of the endoplasmic reticulum and of the Golgi apparatus, removal of channelling, and so on. A recent book (Young *et al.,* 1997) enquires into these correlations.

Buchner's experiment had a double impact: it decreased the vitalist contribution to biochemical thinking by establishing that a fundamental cellular *in vivo* process could be studied *in vitro,* and it initiated a study of the chemical nature of this and of many other cellular processes. And thus the Janus-like nature of Buchner's work was driven home, since these two results are related: vitalist persistence would have made *in vitro* studies impossible, and, in a complementary fashion, *in vitro* studies would have no *in vivo* significance. The abolition of vitalist perspective abolished backward-looking dogma, while the *in vitro – in vivo* correlation opened the view to the future. We can now see that it was the solubility and the persistence or relative stability of intercellular enzymes that abolished notions of a mysteriously guiding, essential vital force and the associated idea of a special kind of intracellular chemistry. The vital force, which had beaten a reluctant retreat into biosynthetic versus biodegradative events (Bernard), into insoluble versus soluble material (Kühne) had finally succumbed to experimental inevitability. Vague notions of protoplasm, comparable to that of the ether in physics, were abolished, and *ferment* in the sense of insoluble material whose activity was life-dependent was replaced conceptually and experimentally by *enzyme*. An amazing byproduct of *in vitro* enzyme persistence was the fact that organized function continued in extracts.

We have to return to Wöhler's urine and to examine its relation to Buchner's alcohol. Both the synthesis of urea and the demonstration of cell-free fermentation were accidental. It bears to examine the impact on the dominance of vitalism of these two very different discoveries. Friedrich Wöhler's 1828 synthesis of urea in the test tube is widely (not universally, a point that is not central here) represented as marking the beginning of organic chemistry, just as Eduard Buchner's test tube conversion of glucose to ethanol is recognized to mark the beginning of modern biochemistry.

Wöhler was clearly aware of the fact that his synthesis had overthrown an assumption, that a special vital force had to participate in the formation of an "organic" compound. There were two consequences, the first well recognized, the second usually ignored: (i) the impressive 19th century growth of wrongly named organic chemistry, begun by Wöhler's work, followed his initiative by emphasizing synthesis (cf. the title of Marcellin Berthelot's influential textbook (1860b) *Organic Chemistry Founded on Synthesis*); (ii) organic chemistry developed by deliberately and consciously doing without biological processes, since the very nature, the properties as it turns out, of organic molecules made this possible: any resemblance of organic synthesis to synthesis in organic material was ignored as irrelevant and, if one had asked a practicing organic chemist, as undoable.

The development of organic chemistry did not abolish vitalism, it simply confined the vital force to processes in the living cell rather than assigning it as a necessary participant in the formation of these molecules. The matter is murky, since biological processes include the synthesis of biological molecules. Organic synthesis was just a small step in the direction of removing from organic molecules an aura of vitalist mystery. Seventy years had to elapse before Buchner's work lifted vital processes from the cell into the test tube, thus initiating what organic chemistry had not attempted: the duplication of a cellular chemical process away from an intact biological system. Buchner's discovery of cell-free fermentation near the end of the 19th century bears a strange analogy to Wöhler's 1828 synthesis of urea: both Wöhler's and Buchner's work demonstrated that no vital force is needed to form so-called organic compounds. The approach of Wöhler's work, which set the tone for the development of organic chemistry, simply demonstrated that the chemistry of organic compounds was such that they could be made outside tissues by more or less drastic methods that clearly had no relevance to vital processes. The approach of Buchner's work initiated the complementary approach, that the organic among the "organic" compounds could be made, again in the test tube, by the very chemical approaches that were used by the living cell. The vital force was shown to be irrelevant to the operation of those cellular chemical processes to which it had willy-nilly been consigned by the flowering of organic chemistry. It was at long last recognized that chemical influences brought about chemical reactions where, before, vitalist forces were considered at play.

It is the sense of surprise about a newly demonstrated halfway house between the living cell and its products that as we saw permitted the likes of Duclaux and Roux to proclaim that a vitalist contribution remained as long as the synthetic methods of organic chemistry could not reproduce the components and thereby the activities of cell extracts. Although very few enzymes have been synthesized since Duclaux and Roux raised their objections, and certainly none of the enzymes of glycolysis, no such vitalist considerations are resuscitated a hundred years later by our inability to synthesize enzymes in the test tube, or by the requirement for the ribosomal machinery to participate in the synthesis of proteins altered via site-directed mutagenesis. Today's unanticipated success can afford to move yesterday's attitude to a sidetrack of triviality. Buchner's experiment, exactly 20 years after Kühne introduced a distinction between enzymes and ferments, dealt the death knoll to this distinction by showing that it did not exist, that in fact all ferments were enzymes. By then Liebig (1803–1873) had been dead over twenty years, and Pasteur (1822–1895), Traube (1826–1894) and Hoppe-Seyler (1825–1895) had all as it were bowed out within a year of each other before the curtain opened on a new, hitherto nebulous land, and Kühne (1837–1900) and Berthelot (1827–1907) were near the ends of their careers. A new era had dawned, and a word, "in yeast", had come into its own as a reminder of contentions centred on an age-old concern with alcoholic fermentation, and after 1837, on the agent necessary for this process.

The scientific study of fermentation has an old progeny. Thus the dissertation by the later mathematician Johann Bernoulli, submitted for public defense at Basel University in 1690 had the title *Dissertatio de Effervescentia & Fermentatione* ("On Effervescence and Fermentation"). It was reviewed by no less a person than Gottfried Wilhelm Leibniz (see Maquet, 1997). Numerous references to wide and to our mind confusing ancient uses of the word *fermentation* can be found in Multhauf (1966). The central early interest in fermentation has been admirably surveyed by Fruton (1972, pp. 23–42). As an example, *Zymotechnica Fundamentalis* was the title of an important book (1697) by Georg Ernst Stahl, the person remembered for using phlogiston "to form the core of his system of chemistry" (Fruton, 1972, p. 33). A hundred and forty years later, right from the demonstration in 1837 that yeast was alive, yeast became the touchstone of vitalist contention, not only because it was readily available, not only because of its intrinsic interest as an agent directly related to the formation of alcohol, but because it was so very easy to look for fermentation. So it was used by Lüdersdorff (1846) and others, by Berthelot, by Pasteur, by many others, and eventually by Buchner. If Pasteur or others had succeeded in obtaining a cell-free active extract from some other microorganism, most of which, as we now know, are easier to open up than yeast, the impact would have been much less dramatic. Buchner's work continued to pursue vitalism's first retreat, from the presumed "organic" properties of organic molecules, to its second refuge, the presumed inscrutable mode of chemical synthesis and behavior in living cells. His work, in turn, set the tone for the development of modern biochemistry that has proved ever more fruitful and ever more valid as the 20th century is proceeding towards its end.

The development of biochemistry was very much slower than that of organic chemistry. Thirty five years had to elapse after Buchner's discovery, and more than a hundred years after Wöhler, before Krebs and Henseleit (1932) showed how urea is made in living tissues. At that time work was proceeding on the unravelling of the details of the chemistry of many other biochemical processes as well. Buchner's zymase, in particular, had undergone a lengthy and at the time not quite completed dissection into unanticipated components, including, as it turned out, as many as twelve enzymes, various essential ions, a coenzyme and a cofactor, and remarkable ramifications into the process of so-called glycolysis.

It is symptomatic of the divergence of biochemistry from organic chemistry that in the classic paper on the urea cycle there is no mention of the much earlier and biologically irrelevant first organic synthesis of urea. By 1932 it had become abundantly clear that the study and understanding of the various biochemical processes had a direct lineage to Buchner's work. More time had to elapse, however, before any remaining beliefs in a vital force could receive yet a further push into the netherworld of irrelevance: by a supreme irony the unraveling of organic chemical mechanisms, achieved independently of biological systems, has demonstrated to the point of obviousness, or if you like of paradigmatic certainty, that biochemical mechanisms are organic chemical mechanisms. Chemistry, and especially organic chemistry, has come full circle. Wöhler's urine has, as it were, come home to roost, and it is Buchner's work that began to build its biological home. Buchner's observation, fortified by the organic chemistry initiated by Wöhler, was a prelude to the immense edifice of biochemistry that has sprouted throughout the 20th century and that continues to sprout with unallayed vigour and unbounded impact on understanding and on application.

A short, lucid, remarkable address from our own days puts Eduard Buchner's achievement into a luminous perspective. This address, given by the 85 year old Nobel Laureate Otto Loewi as President of the Honorary Committee of the Fourth International Congress of Biochemistry, Vienna, September 1958 serves as a bridge between our days and those of Eduard Buchner. At the time of Buchner's discovery Loewi was just finishing his medical studies:

> I was already 24 years old when Eduard Buchner in 1897 showed that sugar could be fermented by a cell-free yeast extract, from which he then prepared "zymase". I vividly remember the tremendous sensation [*ungeheures Aufsehen*] that this discovery elicited, far beyond the circle of biologists and chemists, for instance also in the field of the philosophers, for it once and for all contradicted the thesis of the great Pasteur, that a chemical process as complex as fermentation would only be possible in the living cell. I began [my address] with Buchner's discovery because it, and the method used by him, in the words of a Stockholm committee, opened the way to exact ferment research. The effect on the development of biochemistry was and remains powerful. This is understandable when one agrees with Frederick Gowland Hopkins that the final aim of biochemical research is a sufficient, acceptable description of the dynamics of the living cell. In order to approach this goal it was first of all necessary to isolate and to analyse the individual cell components, especially those that serve as substrates and the factors that act on them, especially the ferments, and to elucidate their successful activity [*Wirkungserfolg*] and the mechanism of their activity. These experiments could of course not be performed with living cells; hence one had to use cells whose struc ture had been destroyed, extracts

> obtained from them and of late apparently intact cell constituents such as mitochondria and their colleagues. Undoubtedly the countless observations obtained with such materials also apply to the living cell, and so one obtained from the beginning of our century a comprehensive and deep insight into the kinds of many fundamental chemical processes in the cell, and into their reciprocal relationships.
>
> LOEWI (1958, my translation)

> How beggarly appear arguments before a defiant deed!
>
> WALT WHITMAN, *Leaves of Grass, Song of the Broad-Axe*

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