BIOTECHNOLOGY

Strategies for Life

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Introduction

1953: In Cambridge, England, two men discover the secret of heredity in the double-helix structure of the DNA molecule.

1973: In San Francisco, two other men succeed in creating the first "chimera" using genetic-engineering techniques.

Between these two dates something extraordinary occurred: men had learned to decipher, spell, write, and even correct the spelling mistakes of life itself. Today, in only a few weeks, a student can "learn to tinker in the laboratory with the very molecule of heredity as if it were a common auto engine," to use François Jacob's expression.

In industrial and university laboratories throughout the world, researchers are trying to get microorganisms to produce substances they would not secrete naturally, to nourish themselves on petroleum spills and other noxious wastes, and to concentrate lean mineral deposits. Biologists, microbiologists, biochemists, and geneticists are exploring the mechanisms that control and regulate every living being on earth, men and animals, plants and bacteria. All of this will have incalculable consequences for the diagnosis and treatment of such mysterious diseases as cancer and thrombosis, the diagnosis and correction of genetic deficiencies of children still in their mothers' wombs, and the improvement of the plant species used to feed mankind. Having explored matter and the infinitely small and the stars and the infinitely large, it is now time to explore "Man, the unkown."

A revolution or a renaissance? Have we not always produced fermented food and drink with the help of microorganisms? Have we not had vaccines since Pasteur? Or antibiotics since just after World War II? And have we not, from time immemorial, crossbred plants?

What has really changed is the possibility of tinkering with the genetic heritage of living matter, of recombining genes, modifying natural organisms, and domesticating microbes. And this has led to the birth of a bioindustry, which is not really a new industry but a complex web of enabling technologies giving new impetus to the conception, orientation, and strategy of traditional industries in such diverse fields as pharmaceuticals and medicines, food processing, agriculture, energy, and pollution control.

Bioindustry is in fact a series of paradoxes. The first paradox: Most major bioindustrial inventions are not the result of directed research but often answers to questions that have arisen in other fields. Thus the enormous vaccine industry stems from Pasteur's research on the refraction of polarized light in crystals. As François Jacob says, "If we had been specifically looking for a tool with which to cut DNA, we would never have found it as did Werner Arber in studying the phage, the restriction enzyme that has become the scissors of genetic engineering." Phages are viruses infecting bacteria, and only a handful of scientists were studying them during and just after World War II. "Purely an intellectual game, a form of folklore of interest to barely ten people on earth!" added Jacob, speaking of a friend who, passing him in the corridor, jokingly asked, "So, how are you getting along with your phage?" And it was exactly from this study of the phages that sprang molecular biology and the "phage" group founded by Max Delbrück, Salvador Luria, and Alfred Hershey. One of their students was a certain James Watson, who, along with Francis Crick, was awarded the Nobel Prize in Medicine for having determined the structure of DNA.

Another paradox: With the biotechnologies, bankers and investors are for the first time putting their money into products that not only do not exist but that no one can yet specify! For, although the electronics industry is the brainchild of research directed and financed by the government and the army, biotechnology owes its existence to private enterprise alone. Silicon Valley has now become Silly Clone Valley. Venture capitalists, investors with risk capital, avid for further "success stories," have in an instant turned from "chips" to "bugs." Wishing to avoid the error of the major manufacturers of vacuum tubes (General Electronic, RCA, Sylvania), which were ousted from the component market for not having negociated the switch to semiconductors in time, businessmen are now preparing for what promises to be a boom in the pharmaceutical and chemical industries, in energy and agroindustry. If, when J.J. Thomson discovered the electron in 1897, investors had proceeded to bet heavily on Jean Perrin, Robert Millikan, or Lee De Forest (inventor of the triode), we would have a good analogy with what has been happening in biotechnology since the late 1970s. And many university graduates, on hearing the sirens' song, have followed the example of Herbert Boyer, Walter Gilbert, or David Baltimore (and many others) in ferreting out capital, founding new biotechnology firms (NBFs), directing foundations, or sitting on boards of directors. Dramatic breakthroughs have been the rule since the very beginning, with Genentech stock being snapped up by Wall Street as soon as it was put on the market in 1980, then rocketing from $35 to $89 in a mere 20 minutes! Cetus beat still another record the following year when the value of its stock hit $115 million on first being offered. Yet perhaps it had all been exaggerated. Research proved long, difficult, and costly, many small companies were stillborn, and still others had to resign themselves to merging with large corporations and to becoming their own research laboratories, concerned above all with profitability. The initial analogy between electronics and biotechnology breaks down on closer analysis. With the electron microscope, memory systems, and data processing, electronics had provided molecular biology with the research tools it needed, but it also supplied a model, with the concept of a program and a code, which could be applied to living matter. And even back in 1943, had not the famed quantum physicist Erwin Schrödinger, in *What Is Life,* compared hereditary material to a coded message?

Contrary to the situation in electronics, biotechnology is not so much an industry in its own right as a set of enabling technologies for other industries to use. Even so, many technical and economic problems remain to be solved in the transfer from the laboratory stage to true mass production. Furthermore, the "raw material" of biotechnology is living matter, idiosyncratic, subject to instability and to the variability of biological laws, which are not always known or predictable. A microcomputer, on the other hand, is subject only to the laws of physics, which served as the basis for its design.

Living matter is subject to necessity, but chance or hazard also plays an important part. Hazard, undestood by scientists to be independent of cause or effect, has, through an erosion of meaning, become an ambiguous word. Over the past twenty years or so, a scientific literature has arisen that attempts to reconcile these two apparently irreconcilable views of the world around us. With varying degrees of success, physicists and historians of physics have produced an amalgam of science and Oriental philosophy bearing such titles as *The Tao of Physics, The Dance of the Elements, The Eye of Shiva.* To biologists, on the other hand, it is simply a matter of proving or disproving the existence of God or some other "higher intelligence." The most characteristic example of this was provided by Lysenko in the Soviet Union, who persisted in refusing to believe in DNA because it implied that men would no longer be able to determine their own destinies – as taught by historical determinism.

Those attending the many seminars held in Paris during the 1950s began to call themselves "Monod-theists," and to a journalist from *Omni* who came to interview him, James Watson declared, "We rewrote the Bible." Richard Axel, for his part, baptized his son Adam. These were jokes, of course, just as was Dali's contention that DNA proved the existence of God. But such whimsy cannot disguise the subtle change that occurred in our thinking and in our language, or mask our apprehension of the universe, which is so frequently commented upon by important scientists who also happen to be profound writers (François Jacob, Lewis Thomas, and James Watson, to mention but a few).

"In Japan we start from a quite different premise," says Kiyoshi Aoki, a professor at the Life Sciences Institute of Tokyo's Sophia University. "To Western biologists, life is matter; to us, who have been influenced by Oriental religions – in which everything, whether stones or bacteria or monkeys or men, has a soul – life is spirit. The Japanese mind shrinks away from the idea that there is a distinction between higher and lower forms of life or that there is a possibility of swaying evolution."

At the University of Tokyo's School of Medicine, a period is set aside each semester in remembrance of the animals that have been sacrificed, and the Hayashibara Company in Okayama has erected a monument to the hamsters that died in its laboratories. A Kyoto businessman has even raised a stele to the microorganisms (yeasts and molds) to which he owed his fortune. No one in Japan finds this surprising. But how would people in France or the United States react if the Institut Pasteur or Rockefeller University built a monument to their mice?

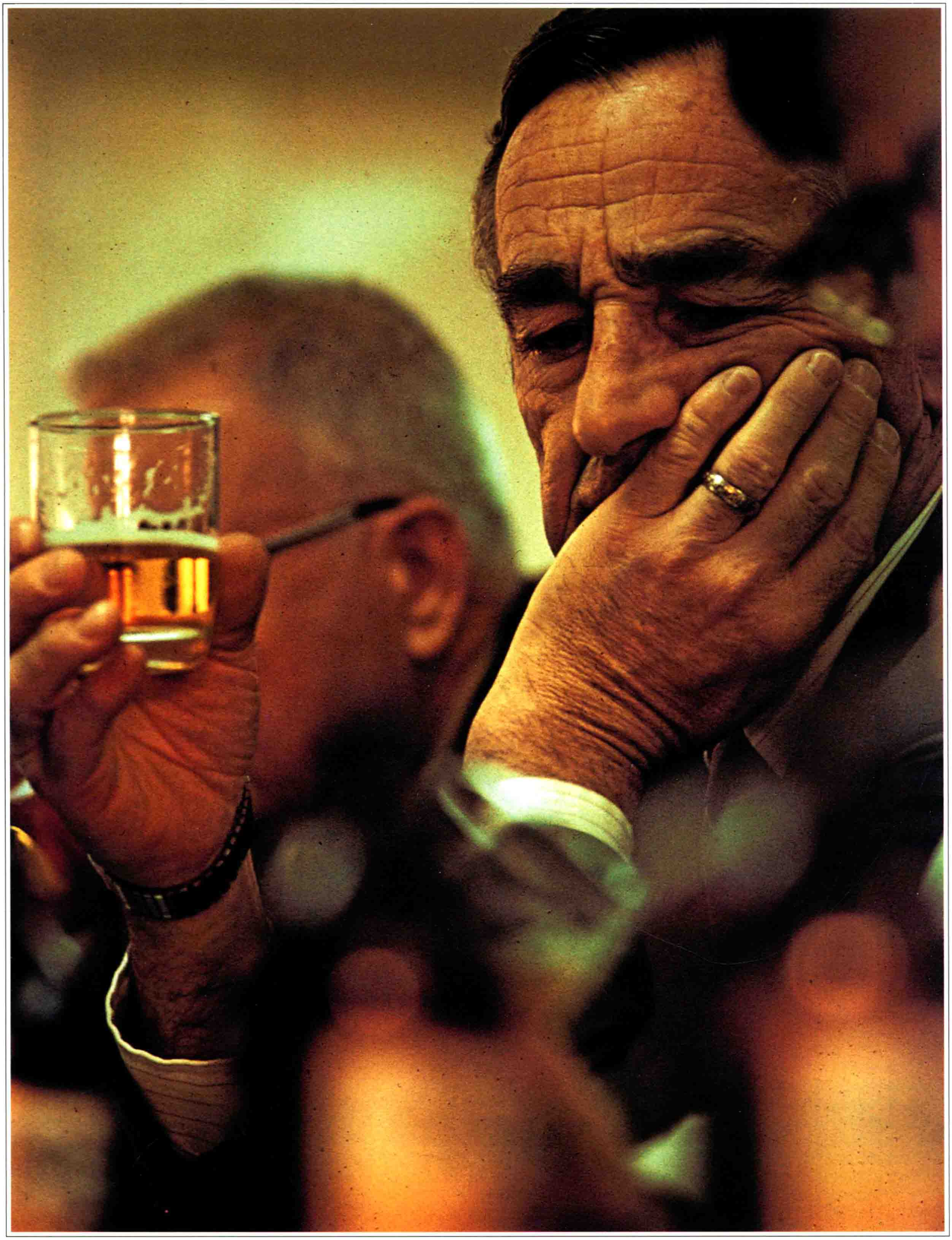
It is only by appreciating this fundamental difference between these two ways of thinking that it becomes possible to understand the odd phrase written by the famed micribologist (and translator of Jacques Monod's book *Chance and Necessity*) Itaru Watanabe, who concluded his remarks on evolution with the words "God is the future of mankind." He seemed to imply by this that transcendence might perhaps be a later stage in evolution, following energy and the Big Bang, matter and physics, life and biology, thought and the brain. In this he is opposed to other scientists, including Nobel Prize Winner in Physics H. Yukawa, who was brought up in China and to whom science is but one part of the infinitude of the universe, which is beyond the scope of science.

One of Watanabe's students and now director of social, natural, and environmental research at the Mitsubishi Kasei Institute, Keiko Nakamura, looks at things less rigidly. From her standpoint, Jacques Monod, if he were still alive, would have to take into account the recent discoveries in molecular biology that no longer make it possible to extend the knowledge we have of bacteria to mankind: "Western science and technology are direct offshoots of

Western philosophy. What we have had urgent need of since the Meiji era and the end of the last century is Western science and techniques, not Western philosophy. Today, young researchers are drawn to the new science and such books as Fritjof Capra's *The Tao of Physics.* Yet this reconciliation with Eastern ways of thinking seems familiar enough to us. The use of life as a raw material for technology naturally enough raises philosophical questions, but Japan is in the habit of assimilating everything – Shinto gods, Buddhism, Christianity – so why not this type of Western philosophy?"

The irruption of the engineer with his mechanisms and systems into a world thought to be immutable has roused mankind's ancestral fears. Though the public has become aware that the "intelligence" of a computer is artificial and consists of man-made programs and that its "memory" is defined by the amount of data it can store, this overly human language had already fostered confusion and imposed a logical sequence resulting in the present model of man: program-code-data. A film like *The Boys from Brazil,* in which Dr. Mengele creates clones – genetic twins – of Hitler, incipient child Führers resembling their "cellular progenitor" feature by feature, raised fantasy to the level of myth... a myth maintained by the heroes of molecular biology who had become legends in their own lifetimes. In *Double Helix,* a book published in 1968 that immediately became a bestseller, James Watson, alias "Lucky Jim" or "Honest Jim," recounted the remarkable history of the double helix "with a little of the innocence and absurdity of children telling a fairy-tale (J. Bronowski, *The Nation,* 18 March 1968, pp. 381-382). "The self-portrait of the scientist as a young man in a hurry," said J. Merton. And young Watson certainly was young at the time of his DNA discovery – only 24. Young, too, are the current superstars of molecular biology – David Baltimore, Mark Ptashne, Walter Gilbert, Leroy Hood, Richard Axel, and Robert Weinberg. This is even one of the outstanding traits of the attraction exerted by these remarkable people who, to parody G. Stent's remarks on Jacques Monod, combine the characteristics of Darwin with those of Prince Charming.

To this seductiveness has been added the golden legend of glory, the avalanche of Nobel Prizes that over fifteen years, has crowned the efforts of many dozens of scientists, and brought fame to research teams everywhere, from Cambridge, UK, to Cambride, US, from the East Coast to the West Coast of the United States and from Europe to Japan. Manna now showers down on these researchers, and over their cradles hover the fairy godfathers of industry and commerce, foundations of every kind, and the purveyors of venture capital. Genetic engineering, protein engineering, microbiological engineering are the subjects on everyone's lips. And as always at the start of any great new scientific adventure, a voyage into the unknown has begun. But with this difference: We ourselves are the unknown.



1

Fermentation : Mysteries Large and Small

Scotch, bourbon, sherry, port, beer and wine, sauerkraut, olives, pickles, yoghurt, cheese – all are everyday Western products of microbology. In the East, Japanese saki and soy-based natto, Chinese and Japanese soy sauce, and Indonesian tempeh are also commonplace foodstuffs. For thousands of years, microorganisms have been helping to make man's diet less monotonous, more spicy and stimulating.

Not until the middle of the nineteenth century and the work done by Louis Pasteur did we learn that beer and buttermilk were not result of a chemical reaction between compounds, but were due do the presence of a microscopically small living cell, yeast. Pasteur also showed that the origin of illnesses could be seen through a microscope as a swarm of living cells, bacteria. All of this unseen life of the cells that feed us and cure us operates through complex fermentation processes that harness minute chemical workers, enzymes.

The prehistory of molecular biology dates back to Pasteur's discoveries. Without the knowledge and mastery of the fermentation process, no product of biological engineering could have seen the light of day: neither antibiotics, nor human insulin, nor perhaps tomorrow the lymphokines, those chemical messengers of immunology. It all began thousands of years ago with the wine that inebriated Noah; the bread that mankind, having been banished from Paradise, had to earn by the sweat of its brow; and the poteen forged in Ireland by the divine smith.

Why does sherry have the aroma of hazelnuts? It is because of the spontaneous growth of certain microorganisms coming into contact with the air as the alcohol-enriched wine matures. The source of the strange flavors of Sauternes and Hungarian wines is the "noble rot" (mycoderma), discovered toward the end of the eighteenth century in the Weingau region. The bubbles in champagne are of carbon dioxide released by a process of secondary fermentation or "frothing" inside the bottle, and the natural autolysis, or self-destruction, of the yeast during the aging of champagne in the cellar. (1) Why does the flavor of beer vary from fruity to flowery, depending on the brand? The brewmaster carefully selects his yeast and its working conditions, such as temperature and acidity, to develop the flavor he wants. Similarly, the various aromas in leavened bread come from the fermentation process, and spontaneous flora leading to secondary changes in the dough after fermentation.

The process of alcoholic fermentation was understood for the first time in 1815 by Louis Gay-Lussac: when subjected to the action of yeast – and considered at the time a physical phenomenon, a sort of spark setting off a chemical reaction – sugars produce ethyl alcohol and carbon dioxide. In 1833, Anselme Payen and Jean-François Persoz isolated the first natural catalyst, an enzyme (*diastase* from malt) capable of liquefying starch and converting it into sugar. But it was not until the second half of the nineteenth century and the work of Louis Pasteur that the idea that fermentation was caused by living microorganisms, rather than being merely the decomposition of inert matter, finally became accepted.

Pasteur was considered "the white knight of science" by René Dubos, and a "soul on fire with an abstract passion no less romantic than carnal love" by Jean Rostand (*Hommes de vérité, C. Bernard, L. Pasteur, C.-J. Davaine,* Stock, 1966, p. 94). Pasteur was still a chemist at the time his master, Jean-Baptiste Biot, gave him the deflection of polarized light as a theme to study. Biot had shown that certain organic substances – sugars and tartaric acids – can, like crystals, deflect light, but also that, in contrast to crystals, they kept their optical qualities in solution. He also made use of the discovery of the German chemist Eilhard Mitscherlich that in the tartar formed during fermentation in wine one may distinguish, along with the large crystals of tartaric acid, the small, needle-shaped crystals of a neighboring acid, paratartaric, or racemic, acid (from the

Beer
Beer, the beverage of gods and heroes from Ancient Egypt to Ireland, from Africa to intertropical America, is undoubtedly the oldest fermented drink in the world. The very word "ferment" comes from the Latin verb to boil," and the general idea persists in some modern popular terminologies (as in the French bouilleur de crus)*. But it was only in the seventeenth century that it became possible to attribute this phenomenon to carbon dioxide. Furthermore, the mysteries of fermentation have marked the religious beliefs of many peoples – the fermentation of bread with the leavening of the spirit, or wine as an initiation beverage from Dionysus to Ibn Arabi and in the legend of the Holy Grail. Today, however, fermentation is no longer a matter of myth but one of fierce industrial competition.*

(1) Lysis is the destruction of organic components as the result of physical, chemical, or biological activity.

Latin word for grape, *racemus*). These two groups of crystals show absolutely the same chemical composition, the same structure, the same specific gravity and the same refraction. Yet, mysteriously enough, a solution of tartaric acid always deflects polarized light, while a solution of paratartaric acid does not. Pasteur observed these solutions under a micrococoscope and discovered that the facets of the polytartrates were asymmetrical: some deflected polarized light to the right, and others to the left. Pasteur was twenty-five years old when he first determined that there was a relationship between optical properties and molecular and crystalline structure – a discovery that was to have considerable as well as completely unpredictable consequences.

A change had begun, and Pasteur slipped from crystallography into biology. It was in this way that, for instance, instead of throwing out paratartrate solution that had been contaminated by mold, he explored its optical properties: the first isomer component (the one that deflects light to the right) is destroyed after a certain length of time, while the second (which deflects to the left) remains alone in solution and provokes intense optical activity.(2)

It was then that a new idea germinated in Pasteur's mind: only living things can produce such asymmetrical, "optically active" substances, and this is undoubtedly the basic distinction that can be made between the chemistry of organic and inorganic matter. "Life, as manifested to us, is a function of the asymmetry of the universe and of the consequences of this fact. The universe is asymmetrical for, if the whole of the bodies which compose the solar system were placed before a glass, the image in the glass could not be superposed upon the reality. Even the movement of solar light is asymmetrical.... Terrestrial magnetism, the opposition which exists between the north and south poles in a magnet and between positive and negative electricity, are but resultants of asymmetrical actions and movements.... Life is dominated by asymmetrical actions. I can even imagine that all living species are primordially, in their structure, in their external forms, functions of cosmic asymmetry" (René Dubos, *Pasteur and Modern Science,* Anchor Books, Doubleday, 1960, pp. 36, 38).

(2) Two isomer compounds are of the same chemical composition and the same molecular mass, but their atomic structures and properties differ.

Biological activity thus depends on molecular structure: this idea was to be brought up again less than a century later by such physicists as Erwin Schrödinger, Niels Bohr, and Max Delbrück and gave birth to molecular biology in the 1950s. As for the asymmetry of the universe, this seems now to have been confirmed by experiments in nuclear physics.

In 1854, Pasteur became concerned with the problems raised by the fermentation of alcohol. At the University of Lille, to which he had recently been posted, he was obliged to devote his efforts to problems of interest to businessmen in that region. This permanent interaction between theory and practice fascinated him all his life, whether it concerned beer, silkworms, or vaccination. One of these businessmen complained of the contamination of beet alcohol during fermentation. Using a microscope, Pasteur detected optical activity, and to him this suggested the presence of living matter. Was yeast in fact something quite different from that complex chemical substance imagined by the leading chemists of the day, J.-J. Berzelius, F. Wöhler, and J. von Liebig? Pasteur had observed that, during the transformation that occurs in the process of milk going sour, the lactic "ferment" that breaks down one sugar molecule into two molecules of lactic acide is nothing more than a swarm of microorganisms. He further showed that their numbers increased rapidly if they were given the appropriate food and that, depending on the ferment, their activity depended on such factors as the acidity (for ferments producing alcohol), neutrality (for lactic ferments), or alkalinity of the medium. He then summarized his ideas and observations in a 1857 "Memorandum on So-Called Lactic Fermentation" containing an early suggestion that "ferments" might also be the origin of infectious diseases. And in reply to a subsidiary question, he added that if the businessman's beet alcohol was contaminated, this was due to the presence along with the ferment of undesirable microorganisms – "infections."

1857 and 1858 were banner years, and it was during this period that Rudolf Virchow published his work on cellular pathology, whose importance we shall see in greater detail in chapter on the cell. It was also at this time that the concept of evolution formed in Darwin's mind. In a speech celebrating the twentieth anniversary of INSERM (Institut National de la Santé et de la Recherche Médicale), François Jacob stated, "A revolution in science is not simply an accumulation of data, a harvest of results, a change in the landscape. It is a change in the way people think, in the way they look at things.... It is a change in vision itself. After Darwin, one can no longer look at living beings the same way. After Pasteur one sees microbes and viruses in infectious diseases where before there were only morbid influences or properties."

In Pasteur's day, all the great chemists were fervent believers in spontaneous generation: that living matter could originate in inorganic matter. Francesco Redi had already fought successfully against this idea in the seventeenth century by demonstrating that worms were not generated from rotting meat but from eggs laid by flies in the meat. This demonstration was accepted for most vertebrates and invertebrates, but there remained one last bastion of spontaneous generation: the microorganisms themselves. Learning of Pasteur's work and his conclusions (that microscopically small beings gave birth to beings similar to themselves), Liebig cried out that one might as well try to explain the underlying currents in the Rhine by the movement of water mills on the Main! It is difficult today to imagine the violence of the intellectual debate Pasteur had to conduct for nearly twenty years to put an end to the myth of spontaneous generation. He finally did so by a series of innumerable systematic experiments and, an arduous task at that time, eliminating all contamination by airborne bacteria. He also showed that, while certain microorganisms need oxygen to grow and multiply, others can do so in the absence of oxygen: so-called anaerobic conditions.

Pasteur thus shed light on the fermentation processes that are at work in similar ways in the production of beer, wine, or saki, bread, cheese, or soy paste. It was this knowledge of fermentation that made it possible for industry to show its mastery in other fields, including the production of antibiotics

Fermentation: Mysteries Large and Small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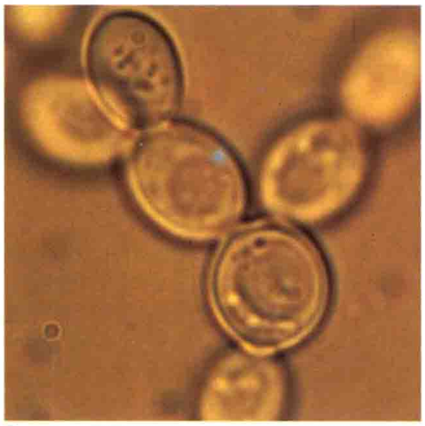
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3


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1, 2, 3. ***Saki***
In the making of saki in Japan, rice is first washed, next seeded with the mold Aspergillus oryzae *(1) to break down its starch, and then inoculated with yeast (2). The boiling activity of fermentation appears on the seventh day (3).*
4. ***Saki***
Early twentieth-century vats used in Japan for the fermentation of saki.
5. ***Pasteur***
In this late nineteenth-century print, Pasteur is seen discovering the laws of fermentation.


5