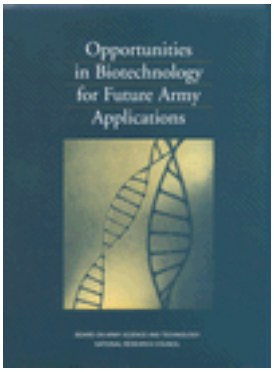

Opportunities in Biotechnology for Future Army Applications

Committee on Opportunities in Biotechnology for Future Army Applications, Board on Army Science and Technology, National Research Council

ISBN: 0-309-50302-7, 118 pages, 8 1/2 x 11, (2001)

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Opportunities in Biotechnology for Future Army Applications

Committee on Opportunities in Biotechnology
for Future Army Applications

Board on Army Science and Technology
Division on Engineering and Physical Sciences
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NATIONAL ACADEMY PRESS
Washington, D.C.

Opportunities in Biotechnology for Future Army Applications
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This study was supported by Contract No. DAAD 19-99-L-0052 between the National Academy of Sciences and the Department of Defense. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number 0-309-07555-6

Library of Congress Catalog Card Number: 2001090219

Additional copies of this report are available from National Academy Press, 2101 Constitution Avenue, N.W., Lockbox 285, Washington, DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu

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Preface

This report has been an extremely challenging endeavor. First, the topic of biotechnology is so dynamic that new developments are being announced almost daily. Consider that the impact of sequencing the human genome in 2000 has already translated into accelerated programs for development of new medicines and of other useful molecules. I believe, as many others do, that biotechnology will continue to develop at a rate that has not been seen since the birth of microprocessor-based personal computers. It was against this backdrop of a rapidly changing science, and an even more rapidly changing translation of science into technology, that the committee addressed the issues in this report.

Second, the scope of biotechnology is expanding so fast that scientists and engineers have difficulty reconciling their perceptions of what is and isn't included; in fact, new and important subdisciplines with linkages to future technologies, such as proteomics, have just emerged in the last few years. A third challenge has been to prepare a report that could satisfy and be understood by an audience composed of both generalists and specialists, as well as by those in the Army who must make the hard decisions on S&T priorities among all technology areas.

The committee examined the basis of new technologies and the probabilities that they could have a future impact on Army capabilities. I believe the report also provides a valuable snapshot of the nature of biotechnology and how its many facets can affect the Army. Although biotechnology is a "moving target," actions can be taken to help track the progression of new biological concepts that will lead to products with the highest potential for Army use.

I wish to thank the committee members for their excellent efforts and the many hours they spent gathering, analyzing, summarizing, and interpreting information, debating the messages that this information contained, and assembling an excellent product.

I would also like to thank Mr. Robert Love, study director, for assembling the committee's findings into this report. His ability to coordinate the genesis and writing of this multidisciplinary report was essential to the success of this project. His patience, dedicated effort, insights, and disciplined approach are much appreciated.

Michael R. Ladisch, *chair*
Committee on Opportunities in Biotechnology
for Future Army Applications

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Acknowledgments

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by John C. Bailar, IOM, University of Chicago. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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FIGURES

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Executive Summary

This report surveys opportunities for future Army applications in biotechnology, including sensors, electronics and computers, materials, logistics, and medical therapeutics, by matching commercial trends and developments with enduring Army requirements. Several biotechnology areas are identified as important for the Army to exploit, either by direct funding of research or by indirect influence of commercial sources, to achieve significant gains in combat effectiveness before 2025.

A short scenario in the first chapter describes several prospective applications for biotechnology developments. The second chapter provides background information on the science and history of the biotechnology industry. The following five chapters discuss research and developments in biotechnology areas that are known to be of importance to the Army and that are within the expertise of the study committee. The final chapter provides conclusions and recommendations.

Although soldiers in 2025 may look much the same as their present-day counterparts, they will be drawn from a society that has been armed by biotechnology with increased strength and endurance and superior resistance to disease and aging. By then, biosensors may be able to detect chemical, biological, and environmental threats of all kinds, bioelectronics components could enable combat systems to survive in high-radiation environments, biologically inspired materials could provide light protective armor for soldiers, and therapies for shock trauma from excessive bleeding could be developed. These are but a few of a myriad of possibilities, some of which may never be developed for lack of commercial incentive, thus challenging the Army to devise ways of influencing their development.

Commercial investments in biotechnological research and development dwarf government investments. To keep pace with developments in biotechnology, Army engineers and scientists will have to become familiar with the language of biology and understand how breakthroughs in the biological sciences will affect the development of new technologies in many disciplines.

STATEMENT OF TASK

The purpose of the report is to assist the Army in planning its science and technology program and to highlight barriers to the development of desirable technologies in the next 25 years. The National Reseach Council (NRC) Committee on Opportunities in Biotechnology for Future Army Applications was asked to accomplish the following tasks:

- Examine developmental trends in the bioscience and engineering industries, including small business involvement and the impact of university and other institutional research activities in biology, biomimetics, and other related thrust areas. Determine what the Army is doing to take advantage of the growth in these technologies. Include, but do not emphasize, medical applications.
- Determine whether trends in research, technology transfer, and commercialization related to the exploitation of advances in the biotechnological industries can be used to predict advances likely to be useful for the Army through the 2025 time frame.
- Identify which bioscience and engineering technologies offer the most potential for Army applications. Consider affordability as well as the likelihood of leveraging commercial research and development.
- Identify critical barriers to the development of biotechnologies with strong potential for Army applications, especially barriers that could be surmounted by appropriate science and technology investments, and suggest ways they might be overcome.
- Recommend research initiatives that could help the Army to exploit promising biotechnologies and engineering developments.

PROSPECTIVE APPLICATIONS

In keeping with national policy and treaty obligations, the study did not include offensive biological weapons. The committee assumed that the Army of the future would

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continue to rely on soldiers to fight and survive on highly lethal battlefields. Although specific combat systems and organizations may change, the Army's fundamental mission of taking and holding ground against a determined adversary will not change. Biotechnology applications relevant to the Army extend well beyond traditional medical areas. As a framework for its evaluation of prospective and applicable biotechnologies, the committee developed a list of enduring Army applications, with topics listed in alphabetical order (Table ES-1).

BIOTECHNOLOGY DEVELOPMENT AREAS

The committee organized its discussion of biotechnology development areas into five broad categories: sensors; electronics and computing; materials; logistics; and therapeutics. The following areas in each category were then identified as providing significant opportunities for the Army:

- sensors: assay analysis; detection methods; chip architectures
- electronics and computing: protein-based devices; biocomputing; biomolecular hybrid devices
- materials: tissue engineering; biologically inspired materials and processes; hybrid materials
- logistics: miniaturization of biological devices; functional foods; biological energy sources; renewable resources
- therapeutics: genomics and proteomics; drugs and vaccines; drug delivery systems

Although not all areas associated with biotechnology could be covered, the scope of the report reflects the committee's best judgment on the most important technology developments to the Army, and in many cases to the other military services, in the next 25 years. The mapping of the human genome and advances in genomics will provide a foundation for many of these developments.

OVERARCHING CONCLUSIONS

The biotechnology industry now surpasses the aerospace industry in market capitalization, research expenditures, and complexity, and the research and development (R&D) budgets of the large pharmaceutical companies dwarf the

TABLE ES-1 Prospective Army Applications

Application	Description
Camouflage and concealment	Biomaterials with stealth characteristics; nonilluminating paints and coatings.
Combat identification	Biological markers to distinguish friendly soldiers.
Computing	DNA computers to solve special problems; biologic models to suggest computer algorithms.
Data fusion	Associative memory and other protein-based devices; artificial intelligence.
Functional foods	Additives to improve nutrition, enhance digestion, improve storage characteristics, enable battlefield identification, reduce detectability; edible vaccines; fast-growing plants.
Health monitoring	Devices to provide feedback on soldier status, enable remote triage, and augment network of external sensors to provide intelligence on chemical, biological, or environmental agents.
High-capacity data storage	Rugged computer memories for individual soldiers.
High-resolution imaging	High-resolution alternatives to semiconductor imagers.
Lightweight armor	Protection for soldiers and combat systems; systems with living characteristics, such as self-repairing body armor.
Novel materials	Biologically inspired materials; biodegradable consumables; genetically engineered proteins; renewable resources.
Performance enhancement	Cortical implants; computer input and display interfaces; prostheses control; sensory enhancement; antidotal implants; gene-expression monitoring; performance-enhancing drugs.
Radiation-resistant electronics	Protein-based components; biomolecular hybrid devices; biomolecular diodes; bio-FETs (field effect transistors).
Reductions in size and weight	Cell-based processes; molecular electronics; biochips; nanotechnology.
Sensing battlefield environments	Laboratories-on-a-chip to detect and identify chemical, biological, and environmental threat molecules on the battlefield; coupling of diagnostic and therapeutic functions.
Sensor networks	Remote sensors mounted on vehicles and carried by soldiers to augment threat intelligence.
Soldier therapeutics	Drugs to counteract shock; genomics-based, directed therapies; optimized responsiveness to vaccines.
Soldier-portable power	Biological photovoltaics; cell-based energy systems.
Target recognition	Protein-based devices for pattern recognition; artificial intelligence.
Vaccine development	Reduced development and production times for small-scale requirements to respond to diseases encountered in exotic locales.
Wound healing	Engineered skin, tissue, and organs; wound dressings and treatments to curtail bleeding and accelerate healing.

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Army's R&D budget. Unlike traditional defense developers, commercial developers in biotechnology are "discovery-oriented"; that is, they are pursuing developments in many directions as determined by the marketplace, which so far is predominantly medical. The Army, however, has become used to managing and influencing R&D directed toward specific procurement objectives.

Conclusion 1. To keep pace with the unprecedented rate of discovery and the anticipated increase in biotechnology developments, the Army will have to establish new, effective partnerships with the emerging biotechnology industry, participate in research, leverage research and developments in the commercial sector, and develop its internal capabilities (organization and personnel) to act on opportunities as they arise.

The biotechnology industry is much less dependent on the military for its existence than other industries with which the Army and other services have routinely interacted. Therefore, the Army will have to use different mechanisms for involving industry in meeting Army needs.

Realizing new applications for biotechnology in a nonmedical area—armor, for example—will require the application of biological disciplines to areas outside traditional medical technology. Although most Army medical applications are similar to (sometimes identical to) civilian applications, nonmedical applications will be much more difficult to identify and influence.

Recommendation 1. The Army should adopt new approaches toward commercial developers to accommodate cultural differences between the government and the biotechnology industry.

Mechanisms that would encourage fruitful relationships between government and industry include contracts that allow businesses to use regular business practices and protect intellectual property rights for nongovernment applications; government funding to mitigate the technical risks of producing prototypes; and minimal requirements for noncommercial, government accounting and audits. These measures would alleviate some of industry's reservations about government contract regulations, restrictions on trade, and the possible negative perception of working with the military on "biological things."

In addition to working relationships with companies, the Army will have to form novel relationships with small and large industry organizations and other government agencies with the same or similar interests. Essential government partners include the National Institutes of Health (NIH), Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC). Making the most of these new relationships will require that the Army develop and maintain its own expertise in bioscience and bioengineering, both to contribute to and gain insights from the biotechnology community and to build on existing expertise and established relationships between the Army medical community and industry.

Conclusion 2. Although medical applications are not the focus of the present study, the commercial markets for medical applications will determine the direction of developments in biotechnology in both medical and nonmedical categories. Engineers and scientists will necessarily become experts in areas that extend biology to other disciplines. To influence developments in Army-significant, nonmedical areas, Army personnel will have to expand their understanding of the role of biology.

Future developments in biotechnology will be accomplished by groups of engineers and biological and physical scientists working together. To leverage discoveries and developments with the highest likelihood of payoff, the Army will need sophisticated in-house expertise in biologic disciplines related to genomics, drug discovery, biosensors, biomaterials, and other specialized areas. Monitoring commercial developments will require broader expertise than is normally required to conduct research. To develop and maintain the needed range of biotechnology expertise, the Army will require both a pool of educated personnel and a strong, in-house experimental program.

Recommendation 2a. To operate effectively in the multidisciplinary environment of future biosystem development, the Army will have to invest in education. In addition to its existing expertise in medical research and development, the Army will need a cadre of science and technology professionals capable of translating advances in the biosciences into engineering practice.

Ideally, these professionals will serve in a mix of permanent and rotating positions. The permanent positions would ensure full-time expertise and continuity of focus on biological developments outside the Army. The rotating positions would enable the Army to interact with key segments of the biotechnology industry and would ensure that the Army remains involved in the latest commercial developments. In short, this cadre of experts would monitor developments, enable the Army to identify new opportunities, publicize Army requirements, evaluate alternative biotechnologies, and otherwise influence the course of developments beyond traditional medical applications to future nonmedical applications.

Recommendation 2b. The Army should conduct a study that focuses on future biomedical applications. The study should explore biological implants, biocompatibility, medical biomaterials, medical defenses against chemical and biological agents, and pharmacogenomics. These will have

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far-reaching implications for future military operations but were outside the range of expertise represented on the study committee.

PRIORITIES FOR RESEARCH

The opportunities in biotechnology discussed in this report are summarized in Table ES-2. Each item includes the committee's recommended investment priority, estimated time frame for realization (i.e., midterm [5 to15 years] or far term [15 to 25 years]), and level of commercial interest. The Army should be especially vigilant in monitoring technologies with high commercial interest in anticipation of industry developments that might be leveraged to meet the Army's needs.

The committee recommended an investment priority of high, medium, or low for each biotechnology area covered by the study. Army investments in research can be catalytic and serve the purposes of both the Army and society as a whole. Commercial technology developments may also go a long way toward addressing Army needs. The committee recommended a "high" investment priority if the technology applications are likely to fill a perceived void for the Army on future battlefields, if the biotechnology appears to offer the most promising avenue toward solving an Army problem, and if the biotechnology is not likely to be developed by industry. A "medium" investment priority was recommended in areas where an Army-sponsored research activity can be used to help open windows on commercial developments; such activities might be conducted as in-house basic research or in the context of cooperative agreements with academia or industry. A "low" priority for investment was assigned to biotechnology areas that should be monitored by an Army expert but do not appear at this time to justify research funding.

Conclusion 3. Five biotechnology areas meet the criteria for high-priority Army investment. These biotechnologies are highly likely to support applications for predicted, Army-unique mission requirements in the next 25 years. In addition, the committee identified four other areas with significant military potential in which focused research investments would help to surmount barriers to developments.

Recommendation 3a. The Army should focus its research in the following high-priority areas in which developments are likely to be accelerated by Army investment:

- three-dimensional (volumetric) memory for rugged data storage
- self-replicating systems for wound healing
- small-scale vaccine production
- shock therapeutics
- vaccine stratification by genomics and toxicogenomics

Recommendation 3b. The Army should support basic research in the following areas to overcome barriers to development:

- determination of target threat molecules for sensors
- proteins for radiation-resistant electronics
- hierarchical design models for bioinspired materials
- structural interfaces for device substructures

Conclusion 4. Most of the biotechnology areas with high potential for the Army are subjects of ongoing research and development by government and/or industry. Continued research in these areas is highly likely to result in near-term advances that will be important for future Army applications. Regardless of the priority assigned by the committee, a biotechnology area may still be important to the Army because opportunities arising from advances in the fundamental biosciences may appear on the horizon with little or no warning. It is also possible that development of a particular biotechnology by a potential adversary would increase its importance to the Army.

Recommendation 4. The Army should monitor near-term developments in all of the biotechnology areas listed in Table ES-2, regardless of the investment priority. The list should be updated to accommodate new opportunities as they arise.

BARRIERS NOT AMENABLE TO RESEARCH

The committee identified several barriers to the development of biotechnologies that could not readily be overcome by more research. These include:

- collection mechanisms for target threat molecules
- ethical and privacy issues that could limit the application of genomics and other biotechnologies and public perception that genetically modified organisms are undesirable
- increasing globalization of development and manufacturing expertise
- certification of biomaterials and nonmedical devices
- length of clinical trials required for development of vaccines

Conclusion 5. Miniaturized, biologically based sensing devices could significantly counter "unseen" threats on the battlefield. Timely sensing of biological, as opposed to chemical, agents will require a broad-based network of both internal and external sensing devices. These devices will require development of micro/nanotechnologies, as well as testing facilities to validate the resulting products. Many of the necessary micro/nanotechnologies will only be developed in response to clearly defined Army (or other DOD) requirements.

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TABLE ES-2 Biotechnology Development Areas

Development Area	Biotechnology	Investment Priority	Time Frame	Commercial Interest
Assay Analysis	Microfabrication/microfluidics	medium	midterm	high
	Affinity reagents	medium	midterm	high
Detection Methods	Optical detectors	low	midterm	high
	Detector arrays of affinity molecules; DNA chips; protein chips	medium	midterm	high
Protein-Based Devices	Optical-holographic high-density memories	low	midterm	medium
	Three-dimensional volumetric memories	high	midterm	low
	Associative memories and processors	medium	midterm	medium
	Artificial retinas	low	midterm	low
	Pattern-recognition systems	medium	midterm	low
	Spatial light modulators	low	near term	low
Biocomputing	Biological models	medium	far term	low
	DNA computers	low	far term	low
Biomolecular Hybrid Devices	DNA-based optical-signal processing	low	midterm	medium
	Biomolecular diodes	low	midterm	low
Tissue Engineering	Cartilage repair and replacement	medium	midterm	high
	Neural bridging	low	far term	medium
	Self-replicating systems	high	far term	medium
	Stem cells	medium	far term	high
	Synthetic biomaterials	low	far term	medium
	Portable, artificial, assisting devices	low	far term	high
Bioinspired and Hybrid Materials	Biologically produced materials	medium	far term	medium
	Biomineralization: organic/inorganic nanocomposites	low	far term	medium
	Hierarchical systems; biocomposites	medium	midterm	medium
Miniaturization Technologies	Microreaction technologies	low	midterm	high
	MEMS-based microfluidic systems	medium	midterm	high
	Biochip architectures	low	midterm	high
	Biological nanotechnology	medium	far term	high
Functional Foods	Genetically engineered foods	low	near term	high
	Edible vaccines	medium	midterm	medium
Biological Sources of Energy	Biological photovoltaics	medium	midterm	medium
Renewable Resources	Renewable fuels	medium	midterm	high
	Nonmedical specialty products based on engineered organisms	low	near term	medium
	Ecological life-support systems	low	midterm	low
Genomics and Proteomics	Genomics data-gathering techniques	medium	midterm	high
	Gene-expression monitoring	medium	midterm	high
	Protein profiling	low	midterm	medium
	Biospectroscopic instruments; terahertz spectroscopy and analysis	low	midterm	high
	Vaccine stratification by genomics and toxicogenomics	high	midterm	high
Therapeutic Drugs and Vaccines	Small-scale vaccine production	high	midterm	low
	Small-molecule and protein therapeutics	low	midterm	high
	Genomics-based vaccine developments	high	midterm	high
	Shock therapeutics	high	midterm	medium
Drug Delivery	Biocapsules	low	midterm	high
	Implantable antidotes	medium	midterm	medium
	Somatic gene therapy	low	far term	high

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Recommendation 5. To influence the direction of commercial developments, the Army should immediately devise strategic and tactical concepts for the detection of target threat molecules and identify Army-unique battlefield requirements for internal (health monitoring) and external (environmental monitoring) sensors. The tactical concepts should address sensing, monitoring, and networking capabilities, as well as interfaces with tactical intelligence systems.

Conclusion 6. The Army can take advantage of commercial developments in gene-expression monitoring and protein-profiling systems and techniques that could lead to devices and technologies for monitoring threats to soldiers in the field (as mirrored via gene expression in response to external stimuli) and provide a foundation for new methods of improving soldier training and performance.

Recommendation 6a. The Army should optimize gene-expression-monitoring techniques for soldier applications, especially for the detection of target threat molecules through toxicogenomics.

Recommendation 6b. The Army should develop predictors of individualized immune responses to vaccines so that they can be tailored to genotypes. It should lead the way in laying the groundwork for the open, disciplined use of genomic data to enhance soldiers' health and to improve their performance on the battlefield.

Conclusion 7. The Army, and the country as a whole, are becoming increasingly dependent on foreign sources for many critical therapeutic materials, such as wound treatments, vaccines, and pharmaceuticals. At the same time, federal and state regulations have restricted both military and civilian research and development in therapeutics. In exceptional circumstances, national defense needs might warrant special dispensation from these regulations, and the Army should have legal recourse for requesting exceptions. For example, in urgent cases, the Army simply cannot wait until developers can meet the extremely high (>99.99 percent) effectiveness demanded by federal regulators and civilian consumers for new therapeutics. In such cases, the development of vaccines and antidotes, quasimedical devices, and biotechnology products for nonmedical uses could be accelerated to meet specific military requirements.

Recommendation 7a. Although the cost of investing in manufacturing infrastructure would be prohibitive, the Army should develop and maintain a database of global manufacturing capabilities, including the biology, processes, and equipment necessary to produce critical therapeutic materials. This database should also include key upstream and downstream aspects of the pharmaceutical industry, such as the status of clinical trials.

Recommendation 7b. The Army should define and petition the government to certify special processes for rapid development and approval of biotechnology applications that meet exceptional Army and other defense needs. The Army and the Department of Defense must have the ability to identify exceptional requirements and expedite the development of products that could potentially benefit soldiers confronted with an urgent threat or special need.

Conclusion 8. Developments in cell biology, immunology, molecular genetics, and genomics have led to new concepts that could greatly improve the safety and efficacy of vaccines and reduce the time and lower the cost of vaccine development and production. The Army must be able to respond to threats with vaccines and antibiotics in weeks rather than years. As the pace of genomics advances quickens, the Army will be hard pressed to take advantage of the many opportunities for providing better vaccines more quickly. Reducing the time involved in clinical trials, which routinely involve large populations, should be a high priority.

Recommendation 8a. The Army should build on its strengths in the development of vaccines by funding new technological approaches that could shorten the time for the development and production of vaccines in response to observed pathogens. These include engineered virus-based vaccines and other genomics developments, such as DNA vaccines, cell-based vaccines, and monoclonal antibodies.

Recommendation 8b. The Army should explore (1) using transgenics to shorten the clinical trial phase for defining toxicity and (2) using pharmacogenomics to shorten the time for Phase III clinical trials.

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1

Introduction

The world in 2025 will be much more crowded, and resources, especially those that require arable land, will be at a premium. Biotechnology will provide a means of feeding growing populations. Therapeutics for treating chronic diseases using biotechnology-derived methods and products will be common, and diagnostics and treatments for cancer, heart disease, and some types of genetic disorders will be more effective and less invasive—at least for nations that can afford them. Vaccines will be available against most infectious diseases. With a better understanding of the basis of life, many of the painful conditions that afflict mankind in 2000 will be preventable.

Many foods will be engineered to provide optimal nutrition and minimize spoilage. Controlling illnesses caused by food-borne pathogens and keeping water supplies safe will be well within mankind's capability. Cost-effective, renewable resources will also have been developed that will compete with nonrenewable petrochemical products. Biotechnology will make all of these things possible.

Box 1-1 describes a visionary combat scenario that highlights possible biotechnology applications for the Army in a not-so-distant future. The scenario described is by no means far-fetched. Potential bioapplications for the Army of 2025 are already on the horizon. Although soldiers in 2025 will look outwardly identical to soldiers today, they will be stronger, have longer endurance, and will be more resistant to disease and aging. The capabilities of future soldiers may very well be augmented in ways that change the nature of individual and unit combat.

Much like the sensors used to detect electronic signatures on the battlefield today, soldiers in the future will wear or carry sensors that can detect signature molecules in the environment, alerting them to changes that may be caused by enemy activity or influence. Sensors small enough to be attached to persons or vehicles, or dispersed by air or munition to distant points on the battlefield, may provide early warning of an enemy intention to pollute the battlefield with chemical or biological agents. Sensors may also enable soldiers to "see" the enemy by detecting trace molecules, similar to the way animals can smell their prey.

Artificial skin could insulate soldiers from environmental extremes, as well as provide frontline treatment for wounds. Uniforms and coatings could contain materials that mimic vegetation to deceive known enemy sensors. Edible vaccines could provide temporary protection against pathogens in exotic locales. Sensor implants could monitor a soldier's health and dispense antidotes to chemical or biological threats, both natural and unnatural.

Small unit operations could also be transformed. Futuristic, "superhuman" capabilities of individual soldiers could enable small units to operate for extended periods of time, carry the fight to remote locales, and endure harsh extremes of climate. Logistical limitations on food, water, and energy in combat could be mitigated by spin-off technologies of the same biological developments that enable burgeoning populations to enjoy high standards of living. Reductions in the soldier's combat load made possible by lighter weight materials and more efficient systems could extend the range and scope of operations so that fewer soldiers will be needed to accomplish a given objective.

Future combat systems of all types will be affected by biotechnology. Regardless of form, fit, or function, these battlefield systems could be constructed of lighter, stronger, biologically inspired materials and structures making them more mobile and capable of surviving the rigors of combat. Combat functions themselves may be modeled on natural, efficient biological processes. It is difficult to imagine any system or function that could not be improved in some way by biotechnological innovations in the next 25 years.

The Army's pursuit of biotechnologies to meet its needs will benefit not only troops and forces, but also society at large. Just as information technologies, which were supported mostly by the military in the 1970s and 1980s, were soon overtaken by commercial development for society at large, new biotechnologies developed for the Army could lead to significant changes for everyone. Lighter and safer

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BOX 1-1
Scenario of Possible Army Applications

Picture yourself driving an Army vehicle similar to an SUV with few amenities. As you move over rough terrain in a Third World country, the vehicle computer, programmed with map data, assists you by smoothing the ride so that you can focus on avoiding shell craters and other obstacles that may not have been anticipated. Your attention is concentrated on keeping the vehicle moving steadily forward to a prominent hill ahead.

Now expand the vision to include explosions on either side of the vehicle and soldiers in the back seat firing weapons. Smoke and haze obscure your vision, but inside the vehicle a heads-up display provides a rapidly changing picture of the battlefield, a continuous stream of information on the location of friendly and enemy troops, vehicles, terrain, weather, and other essential information. In spite of your bioenhanced tolerance to heat, you feel sweat on your face as you try to react to the information displayed while maintaining control of the vehicle.

In your ear the insistent voice of your unit commander reminds you where you are going and what your objective is. It becomes even more difficult to concentrate as you feel your body heat under the airtight garment you wear under your uniform for protection against chemical or biological agents dispersed by the enemy.

Suddenly the display flashes! External sensors carried by adjacent vehicles have detected the presence of a toxic chemical agent. Quickly, a visor in your helmet automatically drops and seals to your face. At the same time, internal medical sensors detect changes in your body, and drugs specifically designed to your needs are automatically administered to calm you and stimulate your reactions. You drive on, secure in the knowledge that your faculties are intact and that your objective lies directly ahead.

But your luck runs out. A guided rocket hits the rear of the vehicle, stopping it dead in its tracks. A gaping hole has appeared on your arm, and your passengers lie frighteningly still. So far you are alive, but as your senses dull, your medical sensors once again detect the emergency. Tourniquets within your uniform tighten around the bleeding limb, and drugs to forestall shock and infection are immediately injected.

Emergency information about the wound is relayed from your health monitor to your personal communications system and transmitted to the central medical unit. Within minutes, the severity of your condition has been assessed and a medical vehicle has been dispatched. You awaken in a hospital and are told you will survive. You can see that new skin tissue has already grown over your wound. You learn that other soldiers in your unit achieved the objective, but as you drift back into sleep you cannot remember actually seeing a single enemy soldier or vehicle. . . .

Thinking back on the operation, you remember that you had filled your vehicle before the battle with a fluid that smelled a lot like wood. The exterior was also sprayed with a coating that would provide camouflage by absorbing infrared and other forms of illuminating radiation. To refresh your memory, you check your personal "black box," the memory module that was issued to you to preserve a record of everything that transpired before, during, and after the battle. It reminds you that you ate potatoes that had been engineered to provide extra nutrition and a distinctive biomarker to enable you to be identified and traced if you had become separated from your unit.

The fusion of so much data and intelligence coming from far-flung sensors required advanced computational algorithms modeled after human neurological processes. Similar models were used to design the efficient communications network that enabled data to be relayed from your internal monitor to the precise location where it could best be used. . . .

As you reflect on the skirmish, you are thankful that the Army had the foresight at the turn of the twenty-first century to recognize the potential of the biotechnologies that make all the difference in combat.

vehicles would consume environmentally friendly fuels. In developing nations, highly efficient foods could extend the food supply, and versatile vaccines could improve survival rates. The Army can use its influence to encourage the development of biotechnologies the private sector might never attempt on its own.

STATEMENT OF TASK

The Assistant Secretary of the Army (Acquisition, Logistics, and Technology) requested that the National Research Council (NRC) carry out a study of enabling biotechnologies for the Army. The study was motivated by a desire to educate the Army about potential developments in bioscience and bioengineering and to assist the Army in planning its science and technology program. In keeping with national policy and treaty obligations, the study specifically excluded considerations related to the development of offensive biological weapons.

The NRC was requested to accomplish the following tasks:

- Examine developmental trends in the bioscience and engineering industries, including small business involvement and the impact of university and other institutional research activities in biology, biomimetics, and other related thrust areas. Determine what the Army is doing to take advantage of the growth in these technologies. Include, but do not emphasize, medical applications.

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- Determine whether trends in research, technology transfer, and commercialization related to the exploitation of advances in the biotechnological industries can be used to predict advances likely to be useful for the Army through the 2025 time frame.
- Identify which bioscience and engineering technologies offer the most potential for Army applications. Consider affordability as well as the likelihood of leveraging commercial research and development.
- Identify critical barriers to the development of biotechnologies with strong potential for Army applications, especially barriers that could be surmounted by appropriate science and technology investments, and suggest ways they might be overcome.
- Recommend research initiatives that could help the Army to exploit promising biotechnologies and engineering developments.

Although the NRC was not asked to focus on specific military or Army requirements, it was asked to suggest novel applications and to pay special attention to technologies that could improve the long-range detection of chemical and biological warfare agents. The study assumed that the Army of the future would continue to rely on soldiers fighting and surviving on highly lethal battlefields. It also assumed that, although specific combat systems and the numbers and configurations of military organizations may change, the Army's fundamental mission to take and hold ground against a determined adversary will not change.

The study intentionally avoided considerations related to the doctrine or organization of future forces and systems, which are subject to change. Instead, the committee focused on applications important to the Army today and applications highly likely to be important in the next 25 years, regardless of changes in doctrine. Future Army applications considered likely are listed in Table 1-1.

The list of prospective applications in Table 1-1 is not all-inclusive: future biobased technologies will undoubtedly create applications that cannot be predicted now. At the same time, some of the applications in the table may represent a long reach for biotechnology, even in 2025. The study attempted to cover biotechnology opportunities ranging from the highly probable to the merely possible, limited only by the Statement of Task and the expertise of members of the study committee.

TABLE 1-1 Future Army Applications for Biotechnology

• Camouflage and concealment	• Combat identification
• Computing	• Data fusion
• Functional foods	• Health monitoring
• High-capacity data storage	• High-resolution imaging
• Lightweight armor	• Novel materials
• Performance enhancement	• Radiation-resistant electronics
• Reductions in size and weight	• Sensing battlefield environments
• Sensor networks	• Soldier therapeutics
• Soldier-portable power	• Target recognition
• Vaccines	• Wound healing

FINDING THE WAY

The starting point for the study was to determine the areas of bioscience and engineering most likely to be associated with the enabling biotechnologies and applications of importance to the Army. In approving the study, the NRC Governing Board Executive Committee directed that a planning group be formed in advance to help determine the disciplines and expertise that should be represented on the study committee.

The planning group consisted of the appointed committee chair, members of the NRC staff and NRC Board on Army Science and Technology, and representatives of the Army. After careful consideration, the planning group determined that committee members should have expertise in biology, biochemistry, biochemical engineering, biocomputing, bioelectronics, microelectromechanical systems (MEMS), biomedical engineering, biomimetics, biomaterials, biosensors, and biotechnology development. The planning group then identified potential sources of information and prospective candidates for the study committee. The list of prospective candidates was used by the NRC as a basis for selecting committee members, with due consideration given to committee balance and conflict of interest.

From the beginning, it was clear that no single committee or study would be able to address all of the areas of biology and engineering that might be important to the Army in the next 25 years. Therefore, the study was focused on areas of biotechnological research and development that the committee agreed could be reasonably translated into useful technologies for future Army applications.

DEFINITION OF BIOTECHNOLOGY

The report addresses possible impacts of biotechnology on the Army, as well as the impacts the Army may have on the identification and development of biotechnologies to meet unique Army needs. Because the field of biotechnology is broad and dynamic, the committee felt it important to develop a definition of biotechnology that reflects the rapidly changing nature of the field and that would be relevant to the content of the report, to the field of biology, from which the developments in biotechnology arise, as well as to the science and engineering contributors to the revolution in biotechnology. Biotechnology will have at least as great an impact in the twenty-first century as information technology had in the twentieth century. Just as we no longer define information technology as telephones and typewriters, the committee did not wish to limit the definition of biotechnology to current biotechnologies.

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The committee defined a biotechnology as a technology with one or both of the following characteristics:

- It uses organisms, or tissues, cells, or molecular components derived from living things, to act on living things.
- It acts by intervening in the workings of cells or the molecular components of cells, including their genetic material.

Although all of the topics in this report fit this definition of biotechnology, not all areas associated with biotechnology are covered in the report. The scope of the report reflects the committee's judgment on topics and applications that will be important to the Army in the next 25 years. Although most advances in biotechnology have been spurred by biomedical research and most near-term developments apply primarily to medical therapeutics and emergency care, the Statement of Task specifically requested that the study not emphasize medical applications. The committee noted that many potentially useful Army applications, such as the implantation of devices in soldiers to augment capabilities or to monitor health, have a "medical" component. For example, insulin delivery systems are very likely to require biotechnology and to evolve into systems with a variety of medical and nonmedical purposes. As bioapplications become common, it will become increasingly difficult to distinguish between the medical and nonmedical domain. The line between drugs that can cure maladies and drugs that can enhance performance is already blurred.

Most bioapplications will also benefit both civilian and military users. Just as separate versions of the Global Positioning System serve both civilian and military users, bioapplications related to the gathering and dissemination of intelligence or to the survivability and mobility of soldiers and systems will have analogues in the civilian sector.

REPORT ORGANIZATION

This report documents the committee's analysis, findings, conclusions, and recommendations. It focuses on areas of research likely to lead to developments of interest to the Army and provides specific objectives for the Army to consider. In Chapter 2 (Biotechnology and the Army), important biological terms are defined, and background information is provided on the biotechnology industry. Chapter 3 (Sensing the Battlefield Environment) describes technologies for biological sensors and detection mechanisms, and Chapter 4 (Electronics and Computing) considers biotechnologies, such as molecular electronics, biocomputing, and biomolecular hybrid devices. Chapter 5 (In Search of New Materials) describes technologies for developing biological, biologically inspired, and hybrid materials. Chapter 6 (Reducing Logistics Requirements) discusses technologies for miniaturization, functional foods, biological energy sources, and renewable resources that could help reduce logistics support requirements. Chapter 7 (Soldier Health and Performance) describes important advances in genomics and therapeutics that could increase the combat effectiveness of future soldiers. Finally, Chapter 8 (Conclusions and Recommendations) provides conclusions and recommendations and summarizes the areas of research on which the Army should focus its attention and resources.

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2

Biotechnology and the Army

This chapter provides a brief history of biotechnology, describes the characteristics of the biotechnology industry, and introduces relevant biological concepts. This background information lays the groundwork for the discussions of developments and applications in subsequent chapters.

HISTORY OF BIOTECHNOLOGY

Although the term *biotechnology* was not used until 1919, ancient civilizations used biological processes to leaven bread, brew beer, and ferment wine. The language of biotechnology used by scientists, pundits, and reporters that has become widespread in the last 30 years began when enzymes were recognized in, and isolated from, naturally occurring bacteria (also referred to as wild-type organisms). Once enzymes could be isolated, scientists could begin to direct the recombination of deoxyribonucleic acid (DNA) and perform genetic engineering, which is the basis of the biotechnology industry.

Typically, only a very small sample of DNA molecules is available naturally. The amplification of DNA in a test tube using DNA derived from wild-type organisms was first performed in 1985 by a method called the polymerase chain reaction (PCR) (see Box 2-1). Using PCR, DNA could be "amplified" to generate a large number of DNA molecules that are exact replicates of the originating material. With large amounts of DNA (measured in milligrams), scientists were able to carry out many studies that would not have been possible otherwise.

The first DNA polymerase isolated and identified was a protein from the thermophilic bacterium *Thermus aquaticus* (abbreviated Taq). The interval between the discovery of Taq and its use in PCR to identify the Hantavirus in 1993 was less than seven years (see Box 2-2). PCR, which can now be carried out in almost any laboratory using an instrument about the size of a shoebox, is no longer the exclusive purview of a few large research centers. In fact, these days experiments with DNA are often projects at high school science fairs. Because PCR has enabled a broad range of investigators to work at the level of DNA, it has democratized accessibility to DNA (Appenzeller, 1990).

PCR can be used in scientifically diverse applications and disciplines. For example, PCR is used to study DNA from long-dead species for tracking evolution, so called molecular archeology. DNA that has survived in ancient tissue for 45,000 years or more can now be amplified to provide large enough quantities for the DNA to be sequenced. Thus, PCR can provide a time machine enabling students of molecular evolution "to retrieve and study ancient DNA molecules and thus to catch evolution red-handed" (Pääbo et al., 1989).

Prior to 1970, gene-directed recombination of DNA was limited to plants and animals and carried out through selective breeding. Today, many plants, animals, and microorganisms have been genetically engineered, and genetic information from one species can even be introduced into a different species. New and beneficial properties can be introduced in a directed and predetermined way, starting with test-tube manipulations of DNA. For example, DNA derived from a human being has been introduced into *E. coli* to create a modified bacterium from which human insulin can be derived.

Some bacteria in nature produce a protein that is toxic to insects. When information that enables the bacteria to make this protein is introduced into a plant, the plant can generate this compound and become disease resistant. The creation of *transgenics* (i.e., plants or animals that carry genes from a different species and incorporate them into their own genetic information) is a major achievement of biotechnology.

The rapid development of laboratory tools and reagents has resulted in an expanding base of knowledge about the molecular basis of biology. Future commercial applications may include the following:

- detection of pollutants in the environment
- diagnosis and treatment of diseases
- development of new materials to replace materials

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BOX 2-1
The Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an enzyme-mediated, *in vitro* amplification of DNA for purposes of analysis. Since about 1985, this method has significantly increased the ease and speed of isolating DNA sequences *in vitro*. Developed by scientists of Cetus Corporation in 1984 and 1985, PCR is an enzyme-catalyzed reaction that facilitates gene isolation and eliminates the need for the complex process of cloning, which requires the *in vivo* replication of a target DNA sequence integrated into a cloning vector in a host organism. PCR is initiated by DNA denaturation, followed by primer annealing; a DNA polymerase and deoxynucleoside triphosphates are then added to form a new DNA strand across the target sequence. When this cycle is repeated n times, it produces 2^n times as much target sequence as was initially present. Thus 20 cycles of the PCR yields a one million-fold increase or amplification of the DNA. Applications of PCR include comparisons of altered, uncloned genes to cloned genes, diagnoses of genetic diseases, and retrospective analyses of human tissue.

Source: Arnheim and Levenson, 1990.

derived from petrochemical sources or to mimic biological processes

- creation of new processes and products for improving foods, fibers, and agricultural processes

The technologies necessary to implement these methods and products are changing monthly. The time line of significant events in biotechnology in Figure 2-1 is a graphical illustration of the accelerating rate of change.

The biotechnology industry, which once consisted of a handful of agricultural and pharmaceutical manufacturing giants, now includes more that 2,000 new businesses and is exploiting products of increasingly sophisticated genetic research. Since the 1970s, when the first commercial company was founded to develop genetically engineered products, the biotechnology industry has grown rapidly in market capitalization and has significantly influenced the quality of the environment and the quality of life. In 1985, approximately 1,500 biotechnology patents were granted; by 1998 the number had increased to more than 9,000. Between 1994 and 1999, market capitalization more than doubled, from $45 billion to almost $97 billion, which is more than the estimated $75 billion market capitalization for the entire defense industry. The summary statistics in Table 2-1 illustrate this astounding growth.

The demand for new drugs is a major incentive for the explosive growth in the biotechnology industry. Several hundred drugs and vaccines are currently in human clinical trials, and hundreds more are in the early development stage. Drug research will be used not only for medical therapeutics, but also to improve sensing and diagnostic capabilities for air and water safety, increase crop yields and improve food quality, provide new techniques for bioremediation of pollutants, enable DNA fingerprinting and forensic analyses, and for many other applications.

These civilian applications overlap with Army needs. Advances in medical therapeutics have obvious applications in mitigating battlefield trauma, healing wounds, and developing vaccines. Sensing and diagnostic capabilities are important not only for detecting the presence of chemical and biological warfare agents, but also for collecting battlefield intelligence on other activities that could affect changes in the environment. Biological sensing and analysis capabilities may also extend to monitoring the health, safety, and performance of soldiers in the field. In general, new knowledge of biology will translate into new devices and new ways of analyzing and solving problems.

Biotechnology is rapidly changing and growing. In 1975, visionaries would not have predicted that a draft of the human genome sequence would be completed by 2000. The developments described in this report may well occur much sooner than predicted. By the same token, midterm and long-term developments may never occur at all. Nevertheless, one trend is clear: Biology will be as important to consumer economics (and the Army) in the next century as physics and chemistry were in the last century.

BOX 2-2
The Hantavirus: A Detective Story

The polymerase chain reaction (PCR) and polymerase from the thermophilic bacterium *Thermus aquaticus* (Taq) have had immediate, beneficial impacts on tracking disease processes by characterizing genetic fingerprints of pathogenic organisms. Hantavirus, which was first isolated from striped field mice near the Hantaan River in South Korea in 1976, is associated with hemorrhagic fevers and renal disease. When a mysterious illness with these symptoms appeared in New Mexico in 1993, the Centers for Disease Control and Prevention (CDC) quickly began to search for the cause. Within 30 days of the first death, viral genes from the victim's tissues had been propagated in large enough amounts so that the DNA could be studied, identified, and sequenced using PCR (Gomes, 1997). The CDC found the virus to be a previously unknown strain of Hantavirus that destroys the lungs instead of the kidneys (Nichol et al., 1993).

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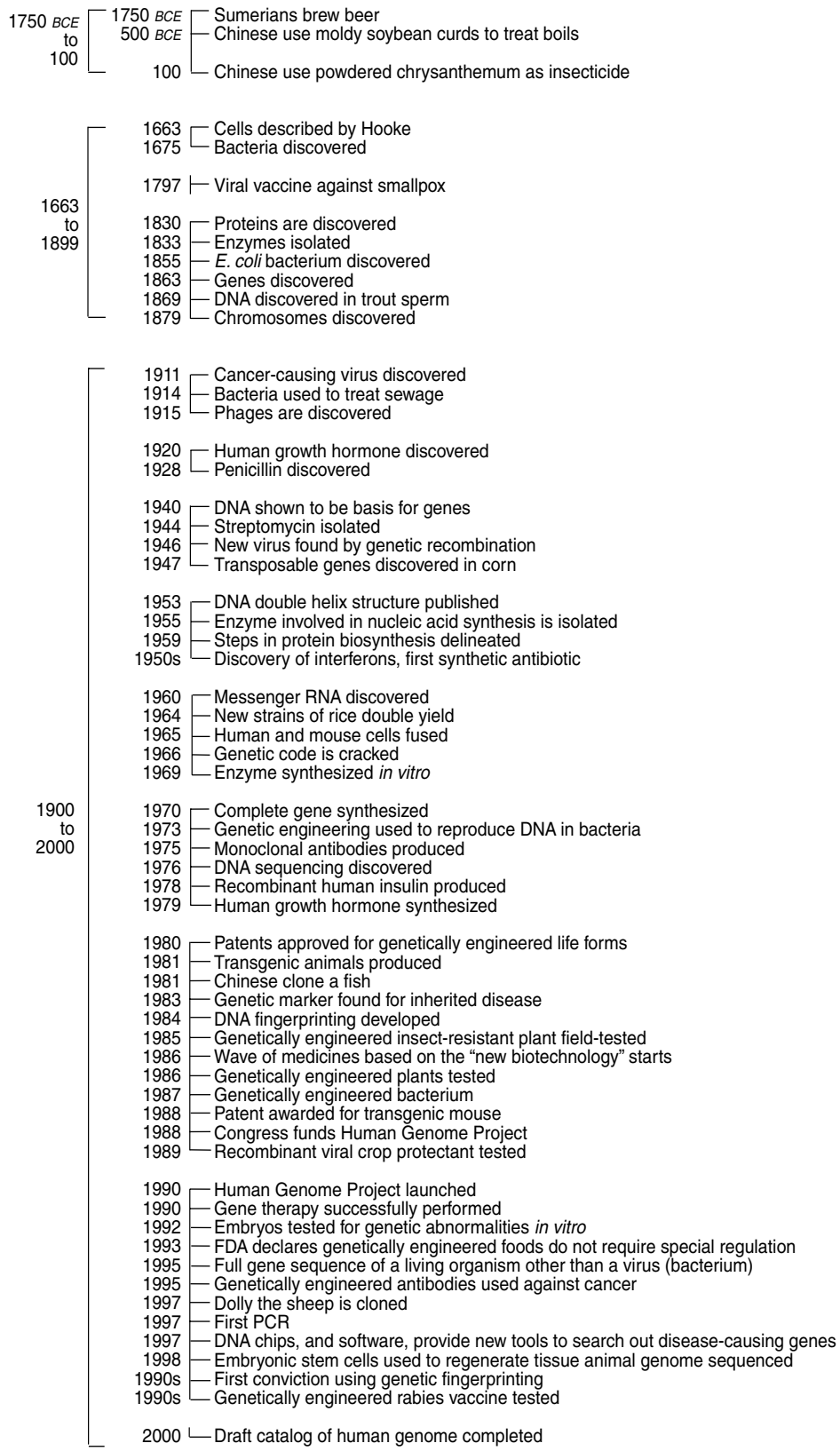

FIGURE 2-1 Timeline of significant events in biotechnology. More than half of the significant events in the past century occurred in the last 20 years. Source: BIO, 2000a.

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TABLE 2-1 Biotechnology Industry Statistics, 1993–1999 (in $ billions)

Year	1993	1994	1995	1996	1997	1998	1999
Sales	5.9	7.0	7.7	9.3	10.8	13.0	13.4
Revenues	8.1	10.0	11.2	12.7	14.6	17.4	18.6
R&D	4.9	5.7	7.0	7.7	7.9	9.0	9.9
Market capitalization	N/A	45	41	52	83	93	97
Number of companies	1,231	1,272	1,311	1,308	1,287	1,274	1,283
Number of public companies	225	235	265	260	294	317	327
Number of employees	79,000	97,000	103,000	108,000	118,000	141,000	153,000

Source: BIO, 2000b.

SCOPE OF BIOTECHNOLOGY

Biotechnology covers all aspects of living organisms, from medicine to agriculture. In the broadest sense, biotechnology is a tool that addresses life itself. Biological principles, through bioprocess engineering, can be used to control the functions of cells or the molecular components of cells, including their genetic material. Through bioprocess engineering, the activities of plants, animals, insects, and microorganisms can be directed to produce bioactive compounds (e.g., compounds that elicit biological activity), therapeutic molecules (e.g., recombinant proteins for treating heart disease and cancer), and other useful materials. The knowledge derived from the study of the genetic characteristics, molecular biology, metabolism, and biology of organisms promises to facilitate the design of devices, software, and genetically altered organisms capable of detecting and diagnosing the effects of pathogens and genetic conditions on human activity.

Biotechnology includes the manufacture of products ranging from food-grade sweeteners to fuel alcohol, as well as the use of chemicals to modify the behavior of biological systems, the genetic modification of organisms to produce new traits, and the treatment of genetically based diseases by the manipulation of DNA. Bioprocess engineering translates biotechnologies into unit operations, biochemical processes, equipment, and facilities for manufacturing bioproducts (Ladisch, in press). Translating the discoveries of biology into tangible commercial products, thereby putting biology to work, requires engineering. Bioprocess engineering is expected to lead to many advances in the future:

- identification of genes, and the protein products that result from them, to prevent or remediate diseases and develop new medicines
- understanding of what proteins do and how they interact to reveal how structure determines function
- production of microorganisms, cells, or animals with new or enhanced capabilities to generate bioproducts, such as new proteins and plants with special characteristics
- development of biological sensors that can be coupled with computers to control bioprocesses and monitor biological systems (including humans)

Central Role of Biology

Assessing the possibilities and probabilities of changing outcomes through biological means or biologically inspired methods requires a comprehensive understanding of biological principles and processes, especially evolution:

> All organisms, and all cells that constitute them, are believed to have descended from a common ancestor cell by evolution. Evolution involves two essential processes: (1) the occurrence of random variation in the genetic information passed from one individual to its descendants and (2) selection in favor of genetic information that helps its possessors to survive and propagate. Evolution is the central principle of biology, helping us make sense of the bewildering variety in the living world (Alberts et al., 1989).

Watson et al. (1992) explained the importance of DNA:

> There is no substance as important as DNA. Because it carries the hereditary information that determines the structures of proteins, it is the primary molecule of life. The instructions that direct cells to grow and divide are encoded by it; so are the messages that bring about the differentiation of fertilized eggs into the multitude of specialized cells that are necessary for the successful functioning of higher plants and animals. . . .[Cells themselves are] tiny expendable factories that simultaneously synthesize several thousand different molecules.

Biomimetics

In a briefing before the committee, the Army program manager responsible for soldier systems (e.g., clothing, electronics, and sensors) expressed the Army's concern over the heavy 92.6-pound load that soldiers must carry into combat (Jette, 2000). Examples from nature suggest that soldier load-carrying capacity and efficiency can be increased. An ant, for example, can bear tremendous loads relative to its weight for relatively long periods of time. In fact, an ant can lift 50 times its weight and pull 30 times its own weight. If this phenomenon were understood, perhaps mimicking the

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ant might lead to solutions that would help soldiers carry even heavier loads.

Biological systems might also serve as models for improving materials for uniforms, particularly by reducing their weight and increasing their functionality. A soldier's clothing must protect against extremes of weather, chemical and biological agents, heat and humidity, and other factors. Many animals cope with similar drastic changes in their environments. For example, an ordinary horse can withstand winter cold and desert heat protected only by hair and its leathery skin. Passive heat transfer alone cannot account for the resistance and isolation necessary to cope with these extreme temperature differentials. Understanding how horses and other animals overcome drastic changes in their environment would be extremely useful.

As a measure of the importance of biomimesis, the Army has declared biomimetics one of its Strategic Research Objectives (primary focus areas for basic research). The Defense Advanced Research Projects Agency (DARPA) has investigated the behavior of insects and other animals in research for the Department of Defense (DOD) (Rudolph, 2000). The principles of design, biosynthesis, and structure-property correlations in "living" materials and systems will be very important in determining new military applications of biotechnology. Thinking in terms of biological systems may not only provide solutions to specific problems, but may also provide clues to future opportunities.

Genomics and Proteomics

Classical approaches to the study of biology have involved biochemistry (the study of proteins in isolation) and genetics (the study of individual genes in isolation). But the examination of an entire genome and its products, a relatively new subdiscipline known as genomics (the study of the genetic material of life), may unlock the secrets of the communication, structure, organization, and interaction of cells and molecules and how they create function. The long-term implications of genomics will present the Army with opportunities and challenges even in the next decade.

Genomics will provide tools for identifying the underlying basis of complex traits, shedding new light on human behavior and performance. It will also help scientists uncover the genetic bases of diseases, such as early-onset Alzheimer's and Huntington's chorea.

The term *proteome* is often attributed to Marc Wilkens, an Australian researcher who proposed the study of all proteins in a genome about five years ago. The subdiscipline of proteomics now encompasses a range of technologies related to the characterization of protein expression, post-translational modifications, and interactions in complex biologic samples (Blackstock and Weir, 1999). Proteomics complements genomics by bridging the gap between genetic message and protein-expression levels (Anderson and Seilhamer, 1997).

As discussed later in Chapter 7, genomics and proteomics have already been instrumental in the development of tools for DNA research, as well as for identifying new materials and applications for biotechnology. However, new applications and capabilities for the Army will require a methodical, systems approach that incorporates a range of scientific and engineering disciplines. Genomics will provide many, but not all, of the answers.

The committee believes that no single entity or institution can change the influence of biology or the trends in biotechnology. The Army can, however, promote development of new products and processes that will be consistent with or specific to its missions and needs. This will require that the Army be fully aware of the synergistic effects of biological tools on the new developments in biotechnology.

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3

Sensing the Battlefield Environment

To accomplish their mission, soldiers must have safe air, food, and water. Unsafe air, water, and food can be avoided and remedied only if the threat is known. Therefore, sensing and detecting a threat must precede intervention to counter the threat. This chapter discusses biological sensors and their role on future Army battlefields.

Since the introduction of chemical agents in World War I, the Army has had to make special preparations to defend against chemical and biological warfare. Future soldiers must also be prepared for this possibility. Just as the Army now depends on electronic and thermal sensors to detect the presence of enemy combat systems, the future Army may come to depend on biological sensors, not just to sense chemical and biological warfare agents, but also to add to its intelligence collection capabilities and knowledge of the battlefield.

Sensing capabilities now packaged in small, lightweight forms that are easy to use and specific for some forms of biological and chemical warfare threats already exist. But these sensors are incapable of responding to all threats, or even to multiple threats; they are designed to perform isolated experiments to detect or verify the presence of known threat agents. Current detection systems and technologies, which may also be suitable for civilian defenses against biological weapons of mass destruction, will have to be adapted to the unique needs of the future soldier environment.

Less sophisticated, less expensive environmental sensors are now used routinely to monitor the environment on a continuing basis for the presence of known hazards. Health monitors, which sense changes in an individual's blood sugar or blood pressure, are also biological sensors. With new developments in biology, and in the applications of biology through biotechnology, other types of sensors and sensing methods will certainly be developed. A challenge for the Army will be to determine the directions of biological research and identify avenues that would lead to sensing capabilities that would increase combat effectiveness.

BIOLOGICAL SENSORS

Biological sensors, or *biosensors,* may be defined as devices that probe the environment for specific molecules or entities through chemical, biochemical, or biological assays. The targets can be airborne, in liquids, or in solid materials. Biosensors may involve any or all of the following functions: detection, capture, concentration, derivitization, and analysis of samples. Biosensor components may have microscale features but may not necessarily be small scale. Ranging from several square centimeters to the size of a computer chip, a small sensor that can perform all of the functions normally carried out at the laboratory bench is sometimes referred to as a "laboratory on a chip."

In general, the response of a biosensor is based on an *assay*, an experiment or test to detect a target molecule. The test is designed based on the known interaction between the target, also known as the *analyte*, and a reagent or organism known to react in the presence of the analyte. Sensing systems generally consist of a recognition element (i.e., a detection element) and a transduction method to translate the recognition into an observable, quantifiable electronic or optical signal. One type of assay, known as an *immunoassay*, is commonly used to detect and identify biological agents, including bacteria, viruses, and proteins.

Different transduction methods based on systems such as mass spectrometry, microcantilevers, miniaturized flow cytometry, and electronic signatures have been demonstrated. Recently, biochemistries have been developed for chemical sensing. Optical methods based on changes in the refractive index have also opened the way for new detection capabilities. Any of these methods, however, will require

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that the Army have an extensive database of potential threat agents and other "target threat molecules" and that these methods be validated for accuracy and robustness under battlefield conditions.

Biochips

Small-scale biosensing devices that incorporate biologically derived molecules that selectively capture specific target molecules are called *biochips*. Biochips use a biorecognition element, such as an antibody (protein) or oligonucleotide, as the reagent to selectively capture and thereby identify target molecules. This process is further described in the section on assay formats later in this chapter.

Biochips may be used in external applications (e.g., to analyze a sample of biological fluids) or in internal applications (e.g., invasive assays, in which devices are temporarily implanted or placed inside the human body). A good example of a biochip is a sensor placed under the skin for detecting blood glucose (see Chapter 7 for a discussion of internal sensing applications and limitations).

Biosensor Network

Small biosensing devices could change the way soldiers "see" the battlefield. Miniaturized, postage-stamp-sized biosensors containing biochips for monitoring the battlefield environment might be worn like wristwatches. In sufficient quantities, these inexpensive, miniature biosensors could provide hundreds of monitoring points for sensing target molecules. Internal biosensors used to monitor physical reactions and other physical parameters could also provide monitoring points.

Most target threat molecules (e.g., chemical or biological warfare agents in liquid or aerosol form) are extremely difficult to detect. The concentrations of samples are very low, and the samples are likely to be "cluttered" with pollen, dust, and other natural biological constituents. The sensitivity of a biosensor also depends on the specific target molecule, some of which are more difficult to detect and assess than others.

The detection of chemical warfare agents is much less difficult than the detection of biological agents, and detectors for chemical agents most likely to be used in combat are under development by DOD. At the same time, for the foreseeable future, effective detection of biological pathogens will depend mostly on the timely reporting and correlation of evidence from the primary health care system, in other words, symptoms reported by affected individuals, rather than on small sensing devices.

For the future, the Army should think in terms of multiple biosensors acting together, rather than single biosensors. Consider that a single miniature sensor system will only be capable of digesting a small amount of air, which may or may not contain enough chemical or biological agent to be detectable. Only a network of biosensors acting in concert and over a period of time would have any chance of detecting a chemical or biological threat at extremely low concentrations.

A network of miniature biosensors carried by soldiers and vehicles deliberately placed throughout a likely battlefield could also have other Army applications. The presence of target threat molecules could provide advance knowledge of enemy presence, activities, or intentions. This biosensory intelligence could be combined with other sources of intelligence, thus providing commanders with new ways of seeing the battlefield and influencing the course of a battle. However, this desirable network of tiny biosensors cannot be implemented until critical development barriers are overcome related to the collection and handling of samples.

ASSAY FORMATS

There are numerous ways of detecting and measuring bioanalytes and using biomolecules to detect other physical-chemical moieties. Typically, measurements of bioanalytes are measured by the identification of a specific aspect of the molecule, such as a marker on the surface of a cell, protein, or nucleic-acid sequence. Each recognition event or method is slightly different in terms of stability, sensitivity, and specificity. In general, cellular assays are fragile and unstable because the cell must be kept intact, if not alive.

Immunoassays

Biologically based technologies for detecting pathogens include nucleic-acid testing and immunoassays. An *immunoassay* is a laboratory or clinical technique that uses the specific binding between an antigen and its homologous antibody to identify and quantify a substance in a sample. Faster, simpler pathogen detection would increase the usefulness of immunoassays.

The Army and DOD have significant programs in place for the development and testing of chemical and biological warfare (CBW) agent detection systems based on immunoassays and, more recently, on toxicogenomics. *Toxicogenomics* refers to methods that measure the expression of DNA in living organisms or cells that have been challenged with a toxin or pathogen (see Chapter 7 for a discussion of genomics applications). In toxicogenomic immunoassays, a predetermined antibody selectively binds a specific antigen in a system set up to indicate the binding event (e.g., by a change in color).

The primary markets for immunoassays are research laboratories and developers of *in vitro* diagnostics. Researchers use a wide range of immunoassay formats, ranging from ELISA (enzyme-linked immunosorbent assay) to western blots. Diagnostics, the largest commercial market for immunoassays, can be divided into two segments: the clinical-laboratory market and the consumer market.

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The clinical-laboratory market uses instrumentation designed for high throughput (the ability to handle multiple analyses quickly) at minimal cost per test in a laboratory environment. The major suppliers to this market are Abbott Laboratories, Becton Dickinson, Roche, and Johnson & Johnson/Ortho. The consumer market is focused on single-use, "point-of-care," disposable products (e.g., pregnancy test kits). In addition to the well-known major players listed above, Unilever, Biosite, and Carter-Wallace are also in this market.

In both markets, the majority of assays are single-analyte assays that rely on polyclonal or monoclonal antibodies derived from the immunization of animals or from cell cultures. In the future, the term immunoassay will probably be a misnomer because detection reagents are likely to be molecular-recognition reagents rather than full-length antibodies. Technologies (e.g., phage display) are currently being used to develop large libraries of antibody fragments that can be rapidly and reliably screened against a variety of target antigens (de Haard et al., 1999). Libraries of smaller protein domains and peptides have also been successfully screened against target molecules to identify recognition reagents with suitable affinities and specificities for use in immunoassay formats (Cannon et al., 1996). Ribosomal or messenger ribonucleic acid (mRNA) fusion-display techniques could construct even larger libraries[1] and provide more rapid screening (Roberts and Szostak, 1997).

In the next five years it will certainly be faster and cheaper to discover "capture-and-detection" reagents via these types of techniques than via conventional immunization techniques. The new reagent molecules will be less expensive to manufacture and may be more stable than antibodies. To allow for lengthy storage periods, the Army should ensure that preservation materials are also developed to extend the shelf life of the reagents.

Methods of discovering binding reagents more cheaply will become much more important as the number of interesting targets increases. Some companies, such as Millennium Predictive Medicine and Diadexus, are focusing on developing capabilities that can be used to define multiple targets of interest for many pathologies and physiologically relevant pathways (see discussion in Chapter 7).

Concurrently, format immunoassays will be moving toward decreasing the size of samples and increasing assay range and accuracy. To achieve this, many firms and academic laboratories are developing promising DNA-array and protein-array technologies. DNA-array technologies have already gained commercial acceptance in the transcription profiling or gene-expression monitoring fields (e.g., Affymetrix, Sequenom, Genomic Solutions); protein-array technologies that would apply to immunoassays are in earlier stages of development.

As a result of these developments, tools and capabilities for immunoassay-type products over a wide range of applications, including those that the Army may use, are likely to become far more capable than they are today. Although nucleic-acid testing techniques will likely remain superior in sensitivity for pathogen detection, immunoassays will be simpler and faster and will respond to a much wider range of challenges.

The issues of size, portability, and robustness for Army applications and field conditions will still have to be addressed. Although only minute (nanogram to microgram) quantities of key reagents, dissolved in buffer, will be necessary, the assays themselves will still require a laboratory bench. Systematic engineering will be necessary to integrate and package assays in easy-to-use kits to address Army needs.

Nucleic-Acid Assays

Nucleic-acid assays use the specific sequences of DNA or RNA of an organism as an identification scheme. This requires isolating the nucleic acid from the background of other molecules and typically performing a DNA amplification step (RNA is usually transcribed back to DNA through a process referred to as reverse transcription) (see Box 3-1). The amplified sequence of the target organism is then detected. In most applications, the amplification step requires enzymatic synthesis of specific sequences of the targeted organism's DNA. PCR (polymerase chain reaction), the most commonly used method of amplification, requires heat activation.

Detection can range from fluorescent detection of the amplified product via electrophoresis, via amplification of an additional nucleic-acid probe, or via the subsequent hybridization of the product to a known matching sequence on an immobilized probe. All of the variations of these methods use a hybridization probe or direct detection of the amplified DNA. Because these assays detect the basic building blocks of biomolecules (i.e., nucleic acids), which produce all of the materials in the biological entity, they are inherently very specific.

Currently, nucleic-acid assays and assay systems (instrumentation and devices) are being developed for pathogen detection, genetic screening, cancer diagnostics, food testing, and other applications. The degree of complexity of the assay is a direct function of the complexity of the sample type. For example, air or water samples may require less processing than tissue or soil samples from which the

[1]In addition to these biologically based approaches, many molecular-diversity approaches use synthetic methodologies. Libraries constructed using synthetic methods include combinatorial chemistry libraries (Terret et al., 1995) and peptide and oligonucleotide aptamer libraries (Buettner et al., 1996; Ellington and Szostak, 1990). These libraries are generally less diverse than their biologic counterparts but have the advantage that selected compounds can, by definition, be synthesized by available chemistries. If synthetic compounds with desired selectivities and affinities are discovered, they could be very stable and readily producible for incorporation into detection systems.

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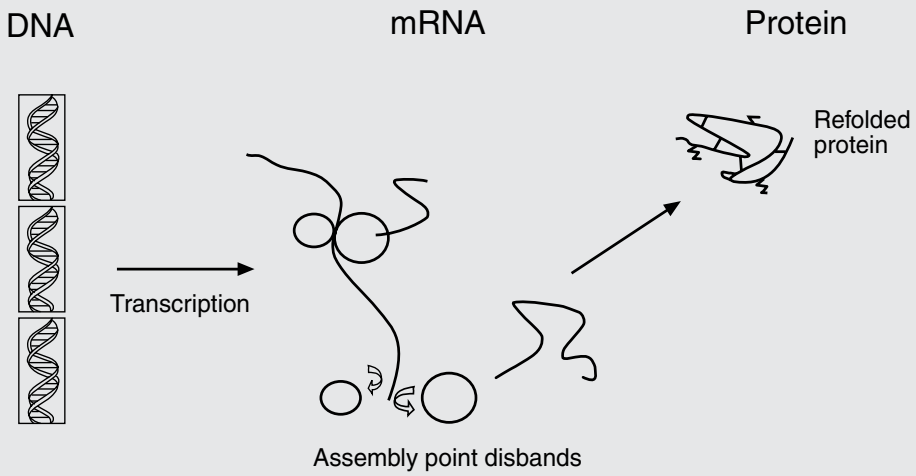
BOX 3-1
Roles of DNA, mRNA, and Proteins



DNA is the informational basis from which living cells derive instructions for synthesizing proteins. Many of the resulting proteins are enzymes that catalyze biochemical reactions from which the cell derives energy or generates other molecules essential to its health and safety. The process normally occurs when the sequence of nucleotides in DNA is transcribed into a complementary, single strand of nucleotides known as messenger RNA, or mRNA. The mRNA provides the instructions by which other components in the cell synthesize proteins. Because not all genes are transcribed (or expressed) but all genes that are transcribed do so through mRNA, the presence of mRNA is an indicator that a gene from the cell's DNA has been expressed.

The DNA from which the mRNA is obtained is sometimes interspersed with oligonucleotide spacers that do not appear in the final mRNA. Because mRNA is used for the cell's molecular machinery to generate the protein, the sequence of DNA (or gene) that corresponds to a protein can be obtained by reverse transcription of the mRNA. The DNA is generated from the mRNA using a test tube (*in vitro*) procedure in which enzymes and several reagents are added to a purified form of the mRNA, which is then used to form the oligonucleotide sequence, or DNA, from which the mRNA was originally generated. The opposite of transcription, this process is referred to as reverse transcription. The DNA obtained in this manner, called complementary DNA, or cDNA, provides a nucleotide that can be further amplified and used to carry out an analytical procedure to obtain the sequence of the cDNA.

Source: Courtesy of Professor Michael Ladisch, Purdue University.

molecule of interest must be extracted. Sample preparation and purification are also critical factors. If the sample-processing challenge can be met, however, the assay will be extremely sensitive and specific.

The development of simple, automated, integrated sample-processing systems coupled with nucleic-acid assays is a critical barrier to their widespread adoption and implementation. Sample preparation is also a critical barrier to the sensing applications important to the Army because the materials to be interrogated by sensing devices are likely to be chemically complex and biologically dirty (see Barriers to the Development of Portable Sensors, below).

A variety of approaches to sample preparation apply. Whether applied on the nanoliter, microliter, or milliliter scales, the principles upon which initial fractionation of the sample is based are similar. The principles of separation that apply to biological molecules are referred to as *bioseparations;* the design of separation protocols is referred to as *bioseparations engineering* (Ladisch, 2001).

DETECTION METHODS

The selection of a particular detection method will affect the overall speed, efficiency, and accuracy of the sensing system. Miniaturization requires detection strategies compatible with smaller sample sizes to increase sensitivities and minimize background effects. The three primary methods of detection that have been used for screening programs are fluorescence, chemiluminescence, and mass spectrometry. Because neither fluorescent nor chemiluminescent methods require fluidic manipulations following an assay, the scale and format of their means of implementation can vary (e.g.,

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plates or chips). Mass spectrometry, however, requires that a sample solution be transported from one location to another. On a microscale, this would require picoliter or nanoliter fluid management via capillaries or microfluidic chips. Therefore, the commercial development of chip-based mass spectrometry has been a high priority.

Two designs of microdevices for microanalysis by mass spectrometry have been developed. In both, electrospray is used for sample ionization and transfer of the analytes from the microdevice to the mass spectrometer. The first design incorporates a capillary-electrophoresis separation channel and a micromachined pneumatic nebulizer to generate a stable sample flow and electrospray. The second is designed for high-throughput infusion analysis in a format compatible with a standard microliter well plate. Samples are deposited into the wells and then analyzed in rapid sequence by consecutive application of the electrospray voltage and pressure to each well. During operation, the microdevice is positioned on a motorized translation stage to ensure that consecutive samples can be analyzed immediately after sufficient data have been collected from the previous sample.

A number of microenabled devices and techniques are emerging in the analytical chemistry industry to address requirements for increased sample throughput and decreased sample volumes. These components, methods, and materials are referred to in the commercial sensor industry as micro total analysis systems (MicroTAS) (see Box 3-2).

Optical Sensors

An optical sensor system also consists of a recognition (detection) element and a transduction method of translating the recognition event into an observable, quantifiable optical signal and, ultimately, an electronic signal. In optical sensor systems, the recognition step is generally based on (1) spectral interactions with the species to be detected (i.e., absorption, emission, and scattering); (2) interaction between the species to be measured (the analyte) and a reagent; and (3) for biological agents, interaction between the species of interest and a receptor.

In some systems, the recognition process involves a combination of steps, such as tags or labels to alter the fluorescent properties of the analyte. Optical transduction schemes typically rely on measurements of spectral intensity (e.g, direct-absorption measurements, fluorescence, FTIR [Fourier transform infrared], and scattering methods, including Raman and surface-enhanced Raman), interferometry (generally based on refractive-index measurements), mass-loading measurements, and stress/strain-induced deformation measurements.

With the emergence of multichannel sensing configurations, signal processing is becoming increasingly important. Fourier transform spectroscopy is an example of the power of signal processing. To meet the demand for field-portable sensor systems, attempts are being made to combine the recognition steps with optical transduction

BOX 3-2
Micro Total Analysis Systems (MicroTAS)

In the late 1980s and early 1990s micromachined fluid components (typically glass or silicon) seemed to be promising for chemical separation devices, reaction chambers, fluid-handling devices, sensors, and detectors. This field of research encompasses relevant advances in microfluidics and has become an important basis for microelectromechanical systems (MEMS) devices and technologies developed for chemical analysis systems. In recent years, the system-level technologies in chemical analysis systems have been increasingly referred to as micro total analysis systems (MicroTAS).

As a result of the developments in MEMS and MicroTAS, various microcomponents for biosensor applications have been demonstrated. However, very few of these components have left the academic setting, been transferred to the manufacturing/commercial environment, been successfully integrated with other key components, are actually for sale, or have been tested with real samples under field conditions.

Although system-level approaches to develop complete instruments are being attempted, the emphasis is still on component-level developments. For example, several groups have begun to explore using the centrifugal forces present in current compact disc players to actuate a pumping/valving mechanism in a molecular biological analysis system. This would be a clever way to take advantage of an inexpensive consumer product for fluid handling. Even if some simple fluid handling developments were achieved, other critical components of a complete system are still in their infancy. For meaningful analyses on real samples, the issues of repeatability, surface characteristics of the device (e.g., hydrophobicity and biocompatibility), reaction chambers, reagent storage, and detection must still be addressed.

Novel materials and methods for fabricating the key components of microsystems are being developed. An example is a hydrogel-based valve that swells/shrinks in the presence of certain salt/pH solutions. This valve has been shown to be very simple and biocompatible. Basic research on the development of microcomponents or nanocomponents have led to the discovery of fundamental principles, such as the very powerful miniature electrokinetic pumping mechanism, a phenomenon that works only in the microscale.

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techniques. Improved optical manufacturing technologies have stimulated the development of miniaturized bulk-measurement systems. The development of fiber-optic and planar-optical wave-guide technologies and the emergence of silicon micromachining technologies have further stimulated the development of microsensor systems. Broadly defined, optical transducer technologies can be divided into the following categories:

- miniaturized versions of bulk optical systems
- evanescent wave techniques
- micro-optomechanical systems

Current sensor approaches rely on assays, require multiple steps, and use multiple reagents, all of which are difficult in field applications and require trained personnel. Therefore, considerable efforts have been made to miniaturize sensor systems that are based on fluorescence approaches, which have demonstrated detection sensitivity in laboratory tests and about which an extensive knowledge base exists.

Recently, direct-detection sensing methods based on evanescent (very short duration) wave and micro-optomechanical transduction techniques have been developed. Evanescent planar-wave sensors based on integrated optic interferometers are capable of detecting biological agents, either directly or indirectly through a two-step labeling process. Chemical sensing techniques using biological receptors to detect chemical species have also been demonstrated. All of these techniques depend on large quantities of analyte.

Unlike other devices that rely on mass change, micro-optic devices detect refractive-index changes. The source of the change can be simple adsorption onto a surface film, the binding of a molecule to a wave-guide surface, or a reversible reaction on a wave-guide surface. The latter would enable active chemistries involving chemical reactions to be used for reversible *in situ* sensing. The electric field associated with a guided optical wave interacts with the target at the atomic or molecular level; thus reactions, even those involving small molecules, can be detected by the charge transfer caused by the reaction.

DNA Chips

Some research has been focused on detecting, verifying the sequence, and measuring nucleic acids, such as DNA and RNA, in a "chip" format (i.e., on a planar surface). With this format, the chip, or surface material, would be inexpensive and easy to analyze. Hybridization is typically used as a source of specificity, and plastic or glass planar structures or beads are used as the chip. Detection of hybridization can be done by several methods, including fluorescent imaging or detection of labeled DNA probes (used by Affymetrix and Synteni), electrical signals, such as conduction, impedance, and electron transfer (used by Clinical Microsystems), refractive index, and others.

Solution-phase detection (used by Luminex) has also been demonstrated as a way to show if and where hybridization occurs relative to the position of the probes on the surface. Methods of making efficient, high-fidelity sequence probes range from the direct synthesis of nucleic acids on the chip (Affymetrix) or surface to the coupling of prefabricated sequences (known or unknown) with various attachment chemistries (Nanogen). Because the hybridization reaction to a surface in a microvolume can be slow, providing turbulence to extend the reaction time is a problem. Using electric fields to move the anionic DNA molecule, demonstrated by Nanogen, has overcome some diffusion issues; however, the electrodes in buffer solutions still undergo electrolysis, producing gases and plating reactions.

The formation of gas from electrolyses has long been an issue, but it can be resolved with proper electrode design (Keim and Ladisch, 2000). Similar principles are likely to be applicable to the microscale, thus providing a starting point for future research. Recent progress has made toward increasing the manufacturing rate and reducing the cost of DNA arrays (Corning, 2000).

The term DNA chips has been used to refer to miniature devices for analyzing molecules, such as nucleic-acids molecules and other biomolecules (e.g., peptides, proteins, carbohydrates), particularly the miniaturization of separation mechanisms (e.g., electrophoresis, isoelectric focusing of peptides and proteins, liquid and gas chromatography, surface plasmon resonance, electrochemical detection). PCR-based applications of miniature reaction chambers are often referred to as PCR chips.

Hybridization between DNA and DNA, RNA and RNA, or combinations including chemical analogues, is a simpler, more robust chemistry than using proteins or other labile biomolecules but is not foolproof. Chip surfaces must be nearly perfectly tuned to perform (or enhance) the recognition event that dictates specificity and sensitivity. DNA-based nucleic-acid assays have been enhanced via microsystems and microfluidics.

Although DNA chip technology is promising, it has many limitations, including reliance of the analytical portions on relatively large instruments (e.g., lasers, photomultipliers, microscopes). Simple correlations of DNA sequence and physiology (phenotype) are still far from adequate for simple, yet highly functional, chips. Proteins, which more closely represent the physiological action that results from life's activities, should be investigated at the system level.

PROTEIN CHIPS

Protein sensors can be divided into sensors designed to detect, and perhaps quantify, molecules in a biological sample from a person, such as a potentially infected soldier, and sensors designed to detect molecules in samples taken from the environment, which could, perhaps, warn of an at-

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tack by chemical or biological warfare agents. Because of market incentives for advances in biomedical applications, most commercial sensors are being developed for samples from people.

Sensors That Detect Proteins in Biological Samples

A protein chip is a device that can detect the presence, and sometimes the amount, of specific proteins in a sample. By 2025, the technology for protein chips to be used for diagnostic purposes in the field might enable a chip to provide information about 20 to 100 proteins in a sample of, say, saliva. A protein chip used for genomic research in a laboratory might provide information on all of the proteins and posttranslational forms of proteins encoded by an entire genome. The development of protein chips will require the development of a number of critical technologies that are currently the subjects of basic and applied research.

Generation of Affinity (Capture) Reagents

DNA chips for monitoring gene expression depend on the fact that single-stranded nucleic acids can be isolated from a solution and unambiguously identified by hybridization. DNA chips are not absolutely perfect in DNA assays; however, analyses must be based on patterns derived from repeated experiments. Proteins consist of polymers of 20 different building blocks (amino acids) compared to only four for DNA (nucleic acids). Agents that can bind to, and thus capture, specific proteins from solution include antibodies, nucleic-acid aptamers, and peptide aptamers. However, no base-base recognition systems have been developed.

For antibodies to be generally useful, methods will have to be developed to produce them in large numbers *in vitro* rather than by immunizing mice or rabbits and collecting serum in a chemically homogeneous form (i.e., with no different glycosylated states). Cambridge Antibody Technology, with pharmaceutical partners, has developed a system for producing homogenous antibodies on a significant scale. Other companies (e.g., Morphosys, Dyax, and Bioinvent) are also active in antibody phage display research. Army applied research at Edgewood Arsenal is focused on analogous methods for chemical biological warfare defense applications.

Nucleic-acid aptamers, described by Ellington and Szostak (1990), are nucleic-acid reagents selected *in vitro*. An aptamer is an RNA or DNA molecule that assumes a particular shape and surface charge distribution that enables it to bind to a target. Aptamers can be isolated against proteins and produced in large quantities *in vitro*.

Peptide aptamers are proteins from combinatorial libraries that consist of fixed scaffolds and one or more variable regions encoded by random sequence DNA (Geyer and Brent, 2000). Peptide aptamers can be isolated *in vivo* and *in vitro* and can be produced in large quantities. Phylos, Inc., is the most significant commercial entity that can scale up production of peptide aptamers, but its interest is in pharmaceuticals. An effective protein chip to meet requirements for battlefield environmental sensors would require the systematic, scaled-up production of many, many different capture agents.

Detection of Captured Proteins

The most sensitive detectors of DNA (e.g., the devices made by Cepheid) depend on amplification of DNA by PCR, by which a single piece of DNA can be amplified 80 percent of the time. In most tests, 10 examples of the same sequence can be detected with near 100 percent reliability. Proteins, however, cannot be currently amplified by *in vitro* methods. Therefore, current devices for detecting proteins are less sensitive. These devices may be based on bioreceptors fixed to their surfaces to capture protein targets. Hence, methods of fixing bioreceptors (which are also proteins) must be addressed by future research (Bashir et al., in press).

Broadly speaking, proteins in a sample can be detected in four ways. One is competition, a technique in which proteins in a sample bind to a capture reagent, in the process bumping off a detectable molecule bound to the capture agent. Various techniques are then used to turn the detached molecule into an amplifiable signal. This technology is not common for protein chips.

The second detection method is to capture the protein and then detect it with a direct or indirect label. In direct labeling, the label (e.g., a radioactive atom or fluorescent dye) is attached to the protein by a chemical or covalent bond (e.g., a fluorescent molecule chemically attached to the cysteine, an amino acid). One could, for example, label all of the proteins to make them fluorescent and then detect their binding by fluorescence at the surface. In indirect labeling, the protein, before or after capture, is bound by another molecule that sticks tightly to it but is not covalently linked to it. This second molecule has some property that makes it detectable; it might be fluorescent, for example, or it might be linked to an enzyme that changes a colorless chemical to one whose color can be easily detected. With either direct or indirect labeling, it is difficult to label proteins uniformly so that the signal is proportionate to their abundance in the sample.

The third detection technique is to use a physical phenomenon that depends on binding of the native, underivatized protein to the affinity reagent. A number of approaches have been tried, such as detecting changes in mass at a vibrating surface, which alters the vibration of piezoelectric cantilevers or the transmission of sound at specific frequencies through surface acoustic-wave devices. Another technique, which involves optical phenomena, is based on changes in reflection of light from a surface caused by changes in the refractive index near that surface in the dis-

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tance (also called surface plasmon-resonance phenomena). The main problem with these approaches is that a large amount of protein mass must be captured to cause an effect. Only the optical techniques appear to be feasible for the highly sensitive detectors that can be used to sense small (submicrogram) numbers of molecules.

The fourth means of detecting proteins is to capture them on a surface and then analyze the bound proteins by mass spectrometry. Insufficient sensitivity is a problem, but some instruments have subfemtomolar (below 10^{-15} mole) sensitivity, and, if a patterned affinity surface is used to capture different proteins, the mass of the bound protein expected to be captured in each spot could be determined.

Systems Engineering and Device Design

Some of the challenges to systems engineering and device design are common to protein detection devices: ruggedness, power requirements, and integration into an efficient system. Another problem, which also applies to other diagnostic devices, is preparing the sample. Proteins differ from each other chemically and have different properties in solution. They must be extracted from the biological sample in a form that protects the properties (e.g., their native structure) the device uses to detect them. Bioseparations engineering must be applied.

Another serious challenge is cost. Materials with the best defined and most manipulatable surface properties, such as silicon, are expensive to fabricate. A disposable or "few-use" device using these materials could be expensive based on current technology. Plastics might be used if they can be integrated with electronic and biological species. The surface chemistry of the plastic would have to be tailored to be compatible with biological species.

All of these challenges are likely to be met by commercial research and development. The Army should monitor developments and apply its resources to adapting commercial technologies to military uses.

Cells on a Chip

Like canaries in a coal mine, living cells have been proposed as sensors for environmental toxins or for pharmaceutical screening. Microelectronic arrays have been used for monitoring metabolism and cellular activity and measuring cellular responses to a variety of agonists. Because several types of cells can grow and thrive on surfaces, the cell/sensor electrode interface can be a transducer of both intrinsic and induced electrical activity. For example, packaged, miniaturized platinum electrode arrays have been used for monitoring and analyzing electrical activity from a spontaneously beating synctium of cardiac cells, providing direct measurement of cell responses to a variety of ion-channel-affecting pharmaceutical compounds (Borkholder, 1999). Impedance measurements of neurons and glial cells were targeted for measurements of cell membrane conductance, cellular adhesion, and motility.

Cells-on-a-chip systems show promise as sensors of easily measured responses, such as beat rate (cardiac cells). Action potentials can be correlated with biologically anticipated responses to stimuli, such as pharmaceuticals and toxins. Issues to be overcome include cell-to-substrate adhesion, signal input/output interpretation, cell culture to cell culture consistency, cell culture chamber designs, increasing the variety of cell types, and keeping cultures alive and well in the field. Research is still at an early laboratory stage, and application of the technology to portable sensing devices is not likely in the near future.

BARRIERS TO THE DEVELOPMENT OF PORTABLE SENSORS

Because target proteins may be diluted in samples that also contain air (or an aerosol) and contaminants, such as spores, the sample sizes that can be obtained are often too small for detection. Enough material must be captured so that proteins (or other "fingerprints") can be identified that are diagnostic for particular pathogens. Other immediate barriers to the development of portable, low-power sensor devices include:

- the need for multiple reagents
- complex systems that are not reliable enough for unattended operation over extended periods of time or operation by untrained operators
- miniaturization without loss of sensitivity
- weight reduction from the current 10 pounds to handheld, wearable systems
- improving specificity to reduce false positives and false negatives

Because there are few incentives for industry to develop devices that can measure proteins in a rugged battlefield environment, research and development will have to be supported by the government. Commercial developers of environmental sensors and many kinds of diagnostic sensors have paid little attention to capture reagents, active surface requirements, sample collection, or sample preparations. Most of the physical phenomena used to detect binding will not be sensitive enough to detect proteins in samples taken from the environment at large. However, current developers are addressing some systems engineering issues (e.g., power consumption).

Combined electronics and biological systems for detecting biohazards have been demonstrated in cultured cell preparations where normal cell activity is modified through exposure to the hazards of interest. Problems have arisen, not just in developing the electronic technology to interface with the biological system, but also with keeping prepara-

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tions viable in the field and providing a stable response in the absence of toxic agents. Demonstrating these systems and exploring their potential use for guarding against specific pathogens will require a great deal more work.

Sample preparation remains one of the most challenging aspects of miniaturizing sensors for chemical and biomolecular analysis systems. Most real-world samples include background material, such as soil, tissue, biological materials, ions, and metals, all of which create differences in sample-processing methods. These processes range from simply diluting the sample to reduce the concentration of a material that inhibits the detection process or assay to elaborate procedures requiring large, sophisticated equipment. Methods used for different samples are rarely interchangeable. For example, the use of focused ultrasonic energy can easily break open a bacterial spore to release nucleic acids. However, too much energy can shear the same molecules and ultimately decrease the analytical sensitivity of the method.

Basic research will be necessary to study the fundamental physics and chemistries of sample purification processes and possibly to develop universal sample processing methods. Research in different types of detection mechanisms may also be useful to the Army. In addition to detection mechanisms with biological components, detection mechanisms might be based on infrared (thermal) signatures or piezoelectric, audio, or magnetic effects.

KEY RECOMMENDATIONS

Miniaturized, biologically based sensing devices could increase battlefield intelligence and significantly counter unseen environmental threats. Timely sensing of biological, as opposed to chemical, agents will require a broad-based network of both internal and external sensing devices. These devices will require development of micro/nanotechnologies, as well as testing facilities to validate the resulting products. Many of the micro/nanotechnologies necessary for these devices will only be developed if the Army provides clearly defined requirements.

To influence the direction of commercial developments, the Army should immediately devise strategic and tactical concepts for the detection of target threat molecules on future battlefields. These concepts should identify Army-unique requirements for internal and external sensing, monitoring, and networking capabilities over and above those being developed for commercial applications and chemical-biological defense requirements.

The resulting concepts are likely to require significant miniaturization of sensors and sensor components that will not be pursued by commercial developers. For this reason, the Army should support basic research that will facilitate the miniaturization of biosensor capabilities for both internal and external applications (see Chapter 6 for a discussion of sensor miniaturization biotechnologies). Other Army research should address specific barriers to development of portable sensing devices outlined above.

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4

Electronics and Computing

This chapter discusses biomolecular electronics and hybrid devices, as well as the relatively new fields in biocomputing. The Army has become increasingly dependent on computers and electronics to achieve high levels of situational awareness, to implement command and control networks, and to support combat systems on the battlefield. In the future, many computing and electronic devices will consist of biologically derived or inspired materials that will increase their usefulness for Army applications.

One problem in discussing research in these areas is the lack of common nomenclature. Molecular electronics is an interdisciplinary field at the interface between chemistry, electrical engineering, optical engineering, and nanoscience. *Molecular electronics* is defined as the encoding, manipulation, and retrieval of information at a molecular or macromolecular level. These functions are currently performed via lithographic manipulation of bulk materials to generate integrated circuits. Molecular electronics (which includes both biological and nonbiological molecules) greatly miniaturizes computer circuitry and provides promising new methodologies for high-speed signal processing and communication, volumetric data storage, novel associative and neural networks, and linear and nonlinear devices and memories.

Biomolecular electronics (also called bioelectronics[1]), a subfield of molecular electronics, involves the investigation of native, as well as modified, biological molecules (e.g., chromophores, proteins, DNA), rather than organic molecules synthesized in the laboratory. Because natural selection processes have solved problems similar to those that must be solved in harnessing organic compounds, and because self-assembly and genetic engineering provide sophisticated control and manipulation of large molecules, biomolecular electronics is a very promising field.

[1]In a more limited context, the term bioelectronics has been used for electronics intended for medical applications.

PROTEIN-BASED ELECTRONIC DEVICES

From 1975 to 1995, scientists in the former Soviet Union participated in a government-sponsored program to leapfrog the West in computer technology by exploring protein-based bioelectronics. Many of the anticipated applications were military and may therefore be important to the U.S. Army, but details remain classified. One of the best-known accomplishments of the Soviet project was the development of biochrome, a real-time photochromic and holographic film based on chemically modified polymer films containing bacteriorhodopsin (Vsevolodov and Poltoratskii, 1985; Bunkin et al., 1981). The published photochromic and holographic properties of bacteriorhodopsin stimulated the international research that continues today. The protein bacteriorhodopsin is representative of the potential that proteins may have for future Army applications.

Bacteriorhodopsin

Much of the research in biomolecular protein-based devices has focused on bacteriorhodopsin (Figure 4-1), a protein discovered in the early 1970s that has unique photophysical properties, as well as thermal and photochemical stability. Natural selection has optimized bacteriorhodopsin for light-to-energy conversion, and the evolutionary process has thus generated a native material that is particularly suited for a number of computer and data-storage applications. Bacteriorhodopsin, which is isolated from a salt marsh archaebacteria called *Halobacterium salinarium* (also called *Halobacterium halobium*) that has existed for about 3.5 billion years, maintains its structure and function in temperatures as high as 140°C, a temperature at which most proteins can no longer function (Shen et al., 1993). With genetic engineering, bacteriorhodopsin can be optimized for specific applications.

Bacteriorhodopsin should not be confused with rhodopsin, the protein in the back of the eye that converts light into

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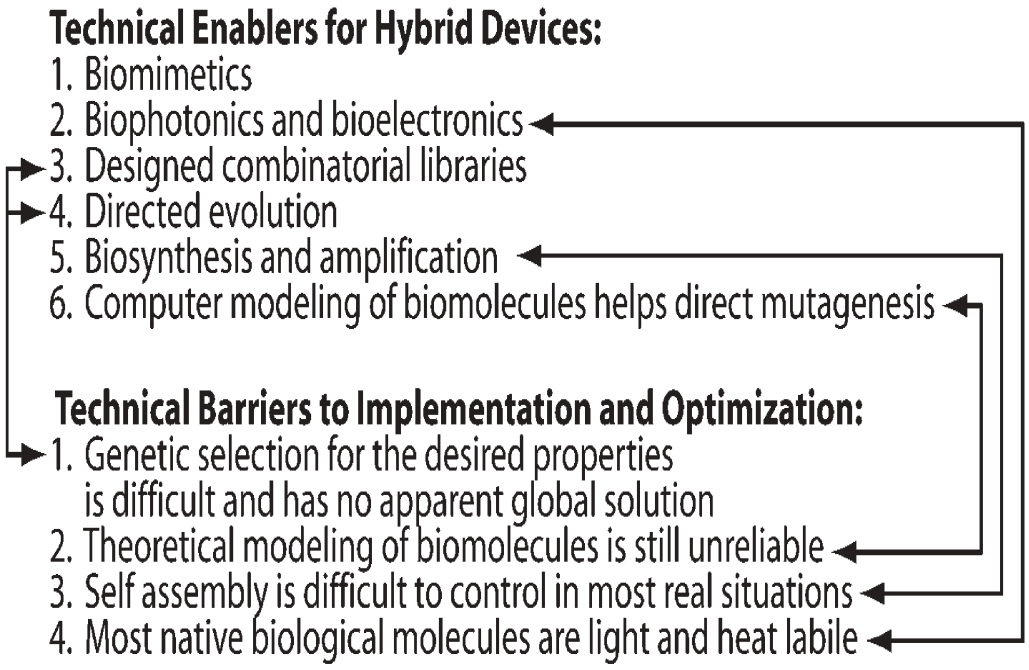

FIGURE 4-1 Simplified protein structures. 4-1a Structure and key intermediates in primary and branched photocycles. 4-1b Structure and key intermediates of bacteriorhodopsin. Note: Maximum wavelengths in parentheses are in nanometers (nm). Lifetimes and temperatures apply to the wild-type proteins only and are approximate.

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a nerve impulse. Rhodopsin and bacteriorhodopsin have some structural similarities but few functional similarities. Rhodopsin is not suitable for device applications because it self-destructs after absorbing light. In contrast, bacteriorhodopsin can convert photons into energy, undergoing structural changes once every few milliseconds, and it can do this hundreds of millions of times before it becomes denatured.

Russian scientists first brought the device applications for bacteriorhodopsin into focus (Bunkin et al., 1981; Vsevolodov and Poltoratskii, 1985). Recently, other photosynthetic proteins have also shown great potential (Boxer et al., 1992; Lee et al., 1997). The principal Soviet investigator, Nikolai Vsevolodov, has since moved to the United States and is now the principal scientist of Starzent, a small start-up company that hopes to manufacture high-density holographic memories. Vsevolodov's recent book, *Biomolecular Electronics*, provides an excellent introduction to the field of protein-based devices (Vsevolodov, 1998).

Scientists using bacteriorhodopsin for bioelectronic devices exploit the fact that the protein cycles through a series of spectrally distinct intermediates upon absorption of light. A light-absorbing group (called chromophores) embedded in the protein matrix converts light energy into a complex series of molecular events that store energy. This complex series of thermal reactions causes dramatic changes in the optical and electronic properties of the protein. The excellent holographic properties of bacteriorhodopsin derive from the large change in refractive index that occurs following light activation. Furthermore, bacteriorhodopsin converts light into a refractive index change with remarkable efficiency (approximately 65 percent). The protein is 10 times smaller than the wavelength of light, which means that the resolution of the thin film is determined by the diffraction limit of the optical geometry rather than the "graininess" of the film. Also, bacteriorhodopsin can absorb two photons simultaneously far more efficiently than other materials. Because of this capability, bacteriorhodopsin can be used to store information in three dimensions by using two-photon architectures. Finally, bacteriorhodopsin was designed by nature to function in high temperatures and intense light, a necessary requirement for a salt marsh bacterial protein and a significant advantage for photonic device applications.

Bacteriorhodopsin can be genetically engineered to do many different tasks. It can be modified by both random and site-directed mutagenesis, which has opened up opportunities to use this protein in biomolecular-electronic devices. Indeed, some applications have used the protein as a template into which new functionality has been programmed via genetic engineering. When viewed from this perspective,

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bacteriorhodopsin is no longer a protein functioning as a photonic device but a complex peptide that can be modified to do whatever one might be clever enough to program into it. It then becomes a prototype for numerous protein-based devices.

Optical-Holographic and Three-Dimensional Memories

One of the most successful applications of bacteriorhodopsin has been in the development of holographic and volumetric three-dimensional (3-D) memories. The holographic memories take advantage of the large change in refractive index that occurs upon formation of the *M* state (see Figure 4-1). Thin films of bacteriorhodopsin can generate diffraction efficiencies of about 8 percent, which is more than enough for holographic data storage (Birge, 1992). In addition, because mutants have enhanced its holographic properties, bacteriorhodopsin is competitive with photorefractive polymers but far less expensive (Hampp et al., 1994). Finally, both thin and thick films can be prepared using bacteriorhodopsin in whatever concentration is optimal for the optical architecture. This flexibility is the key to commercially competitive holographic data-storage systems.

Holographic memories are considered by some to be 3-D memories because the data are stored as a function of *x*, *y,* and θ (the angle of incidence of the write beam). Because this angle is limited by the laws of diffraction to a fairly narrow range, holographic data storage cannot take full advantage of the volumetric storage medium. True 3-D memories can be made, however, by using proteins. One example is the branched-photocycle memory developed at the W.M. Keck Center for Molecular Electronics at Syracuse University (Birge et al., 1999). This memory stores from 7 to 10 gigabytes (GB) of data in a small $1 \times 1 \times 3\text{-cm}^3$ cuvette containing the protein in a polymer matrix (Figure 4-2). Data are stored by using a sequential pair of one-photon processes, which allows the use of inexpensive diode lasers to store one bit into the long-lived *Q* state. The read/write/erase process is fairly complicated, and the reader is referred to Birge et al. (1999) for a detailed description.

The Army Land Warrior Program is scheduled to provide each combat soldier with a wearable computer to assist with the processing of sensor and targeting data, situational awareness displays, and communications. As the use of graphical formats to facilitate the assimilation of information in real time increases, the Army will have a growing need for computer memory capacity on the battlefield. In principle, an optical 3-D memory can store roughly three

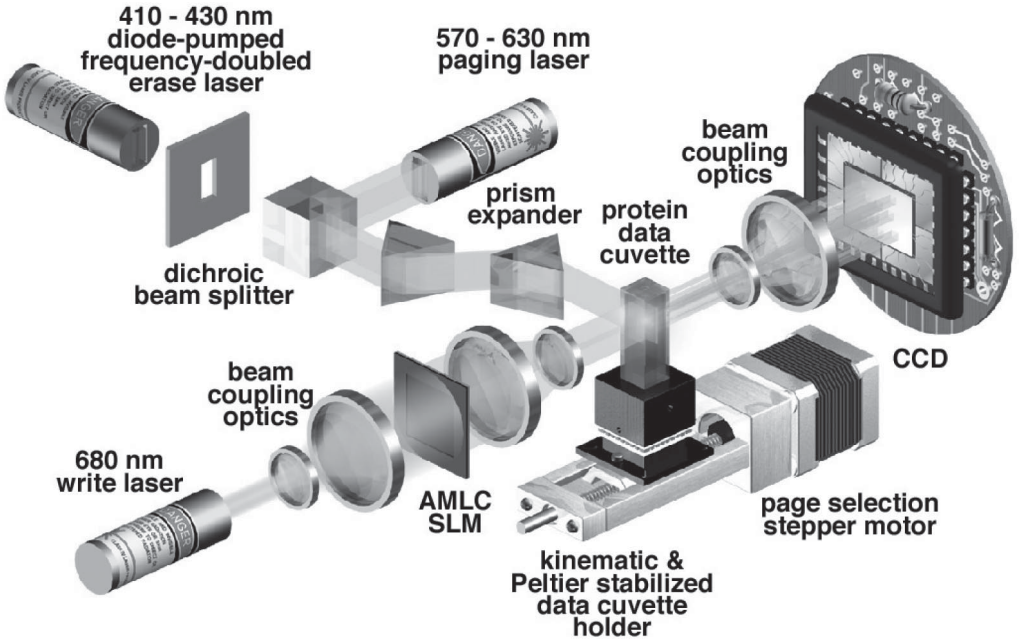

FIGURE 4-2 Schematic diagram of a protein-based, 3-D memory capable of storing between 7 and 10 gigabytes of digital, error-corrected data in a rugged $1 \times 1 \times 3$ cm^3 polymer cuvette. These inexpensive cuvettes can withstand virtually any condition that a human can withstand, including submersion in water for extended periods of time. Selected components are labeled as follows: AMLC SLM = active matrix liquid crystal spatial light modulator; CCD = charge-coupled device.

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orders of magnitude more information in the same size enclosure than a two-dimensional optical disk. In practice, because of limitations in optic reliability, the improvement is an approximately 300-fold increase in storage capacity, which is still significant.

Protein-based memories have an additional advantage in that the memory medium is extremely rugged. The 10-GB data cuvettes used in the 3-D memory described above can withstand substantial gravitational forces and are unaffected by high-intensity electromagnetic radiation and cosmic rays. Another important advantage of bioelectronic memories is low cost. Protein-based polymer cuvettes for 3-D data storage can be manufactured for only a few dollars. They are also lightweight and insensitive to external moisture; they can even be submerged under water for months without compromising the reliability of the data.

Protein-based polymer cuvettes would be a suitable memory medium for troops to carry with them into harsh environments, although extreme temperature can pose problem (boiling water will destroy the data). Directed evolution is currently being studied as a way of improving the thermal capabilities of bacteriorhodopsin.

3-D data storage systems will be developed for the commercial sector, but the Army will need to optimize them for the unique conditions that apply to military environments. Thus, much of the cost of development will be borne by commercial developers, but optimization for military uses must be carried out by the Army. For example, 3-D protein memory cuvettes are already being designed and optimized for archival data storage in office environments; many millions of dollars have been spent on this research and development. Current designs for data cubes are rugged but not rugged enough for possible soldier uses in military environments. In addition, although commercial devices will be inherently resistant to high levels of electromagnetic radiation, they will not be optimized for this important characteristic.

Genetic engineering has been used to create bacteriorhodopsin mutants with enhanced materials properties. For example, some mutants have enhanced the holographic properties of the protein by producing an *M* state with an extended lifetime; others improve the branched-photocycle memory by enhancing the yield of the *O* state (Gergely et al., 1993; Hampp et al., 1992; Miercke et al., 1991; Misra et al., 1997; Zeisel and Hampp, 1992) (see Figure 4-1). The challenge for materials scientists is to predict a priori which amino acid sequence will create or enhance a specific protein property. At present, most genetic engineering for materials applications is done by trial and error, which reflects the complexity of protein structure and function and the lack of satisfactory molecular modeling tools. It is hoped that continued theoretical research will yield computer programs with predictive capabilities comparable to the SPICE software packages that have become a cornerstone for integrated circuit design.

Associative Memories and Processors

Associative memories take an input data block (or image) and, independently of the central processor, scan the entire memory for the data block that matches the input. In some implementations, the memory finds the closest match if it cannot find a perfect match. The memory then returns the data block in memory that satisfies the matching criteria or returns the address of the data block so contiguous data can be accessed. Some memories simply return a binary bit indicating whether the input data are present or not. Because many scientists believe that the human brain operates in a neural, associative mode, it is possible that only large-capacity, high-speed associative memories will be capable of leading to genuine artificial intelligence. Researchers have implemented the neural computer memory designed by Paek and Psaltis (1987) using thin films of bacteriorhodopsin as the photoactive holographic media (Birge et al., 1997). The optimization of the holographic properties of the protein via genetic engineering is an attractive aspect of using bacteriorhodopsin (Hampp et al., 1994).

Large-scale associative memories and associative processors are considered to be critical components in the development of artificial intelligence. The Army also has pressing needs for processing intelligence and sensor data in visual and other formats from multiple sources in real time. For this reason, important Army applications may be to use associative-memory capability for data fusion and high-speed identification of friend or foe. Rapid, confident decisions could be made if associative processors could be programmed to carry out parallel searches for multiple characteristics. Protein-based associative memories could be implemented in rugged, computer-card format and used in the field to assist in complex decision-making processes. An example of a proposed memory system is shown in Figure 4-3.

Artificial Retinas

Japanese researchers were the first to develop protein-based artificial retinas (Miyasaka et al., 1992). These retinas displayed excellent motion sensitivity because of the inherent differential responsiveness of oriented films containing bacteriorhodopsin. In subsequent work, the protein films were integrated with charge-sensitive semiconductor circuitry to provide for higher resolution (Chen and Birge, 1993), but this approach was not successful until methods were developed to prevent the protein and the semiconductor surfaces from cross contamination (Tan et al., 1996). Although protein-based artificial retinas are not significantly better than semiconductor versions (i.e., image sensors), they have the potential to be manufactured for a small fraction of

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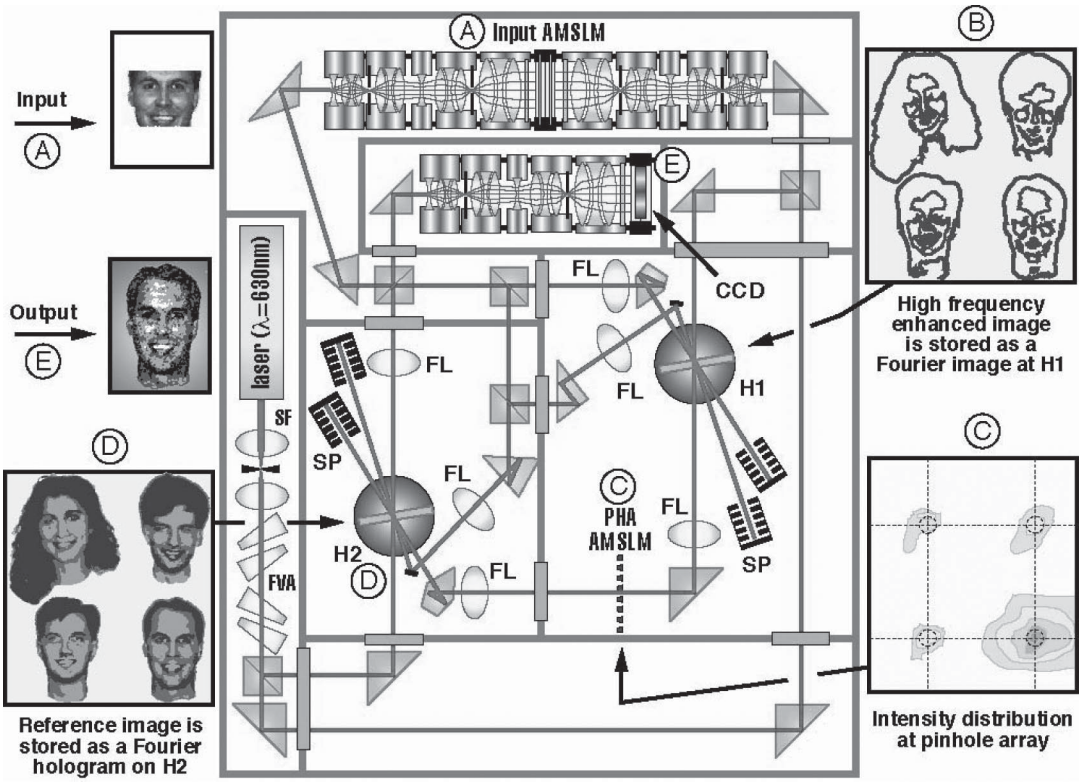

FIGURE 4-3 Schematic diagram of a Fourier transform holographic (FTH) associative memory with read/write FTH reference planes using thin polymer films of bacteriorhodopsin to provide real-time storage of the holograms. Note that a partial input image can select and regenerate the entire associated image stored on the reference hologram. Although only four reference images are shown, an optical associative memory can store many hundreds or thousands of images simultaneously. This memory can also work on binary data by using redundant binary logic, and a small segment of data can be used to find the page with the closest largest association with the input segment. Selected components are labeled as follows: AMSLM = active matrix spatial light modulator; CCD = charge-coupled device; FL = Fourier lens; FVA = Fresnel variable attenuator; H1 and H2 = protein-based holographic films; PHA = computer-reconfigurable pinhole array; SF = spatial filter; SP = beam stop.

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the cost of semiconductor versions. Nevertheless, they will require much development before they will be competitive. Commercial production of both types of imagers will be dominated by packaging cost.

Artificial retinas are capable of providing nearly diffraction-limited performance and so have great potential for Army high-resolution imaging applications. An artificial retina integrated into a highly sensitive motion sensor, could be incorporated into rugged, inexpensive surveillance pods to monitor enemy movements from a distance. Coupled with an associative processor, these retinas could provide a capability for *in situ* friend-or-foe determinations.

Pattern-Recognition Systems

Pattern-recognition systems and associative memories have much in common, including that both systems require a holographic material that is sensitive, is read/write capable, and can operate at or near the diffraction limit. Polymer films of bacteriorhodopsin are the only thick films currently available with these characteristics.

German scientists have used holographic thin films of bacteriorhodopsin to make pattern-recognition systems with high sensitivity and diffraction-limited performance (Hampp et al., 1994). A commercial device is available in Europe that is capable of reading paper currency at high speeds, identifying the country of origin and face value, and also identifying counterfeit bills that have previously been read and loaded into the optical reference data banks. These systems are possible because of the remarkable holographic properties of D96N, a mutant of bacteriorhodopsin (Hampp et al., 1992). This site-directed mutant has both an improved diffraction efficiency and an intrinsic frame rate that permits diffraction-limited performance at optimal video rates for

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high-speed pattern recognition. The Army should consider use of these systems for target recognition and friend-or-foe identifications.

Spatial Light Modulators

Spatial light modulators are available in many forms, from threshold devices, which are very simple, to complex optical systems, which impose data on a beam of light. Bacteriorhodopsin has long been used as the photoactive element in spatial light modulators; in fact, this is one of the most successful commercial applications of this protein (Birge, 1992; Birge et al., 1990; Bräuchle et al., 1991; Oesterhelt et al., 1991; Vsevolodov, 1998). At present, standard and holographic spatial light modulators can be purchased from companies in Germany, Israel, and the United States. The Army currently uses this technology in nondestructive testing systems for inspecting artillery and tank ammunition. The apparatus makes use of the diffraction-limited performance of bacteriorhodopsin thin films to achieve a real-time measurement resolution of 0.005mm over a working distance of 25cm (Stuart, 2000).

Biomolecular Hybrids

Hybrid diodes operating on the principle of photosynthesis are described in Chapter 6 in the section on biological photovoltaics. Other protein-based biomolecular devices include bioFETs (field-effect transistors), which may provide unique architectural opportunities in telecommunications applications, and devices using the photoreactive properties of DNA. BioFETs may enable higher speed operation than conventional transistors, and spintronic injection of semiconductor lattices could produce coherent carrier dynamics, which could be useful for broad-band, fiber-optic converters, and multiplexers. Advances in photonics for telecommunications and radar-signal processing will depend on being able to modulate the phase and intensity of an optical signal. This ability may be enhanced using DNA.

DNA-Based Optical-Signal Processing

Optical-to-optical interactions based on the photorefractive effect and bacteriorhodopsin offer new and potentially more effective alternatives to crystalline materials such as lithium niobate. Solid-state, single-crystal materials are effective but tend to have limited performance capabilities and to be expensive and relatively inflexible in terms of integration with other devices and materials systems. Electrical-to-optical interactions, which rely on the linear electrooptic effect, are becoming more important as means of modulating optical signals at very high speeds and low power.

Recent work performed jointly by Dr. Thomas L. Netzel at Georgia State University and Dr. Bruce Eaton at North Carolina State University suggests that charge migration in DNA may provide the basis for a new class of photorefractive and electro-optic material systems (Rawls, 1999). Netzel and associates have suggested the use of self-assembled arrays of DNA duplexes on the surface of highly sensitive optical wave-guide arrays as a possible photorefractive medium. The approach is based on the attachment of photoactive and redox-active chromophores to 2′deoxyuridine and 2′deoxyadenosine nucleosides. The former would be photoreduced, resulting in electron migration; the latter would be photo-oxidized, providing for hole migration. One of the goals of the proposed research would be to evaluate the effective index change resulting from migration using highly sensitive optical wave-guide interferometers. Assuming that the induced index difference is sufficient, this approach might lead to a very fast optical-to-optical modulation mechanism.

Commercial incentives are strong for reducing the cost of optical-to-optical interactions in the commercial telecommunications market, and the Army's strategic communications infrastructure will be a prime beneficiary. The development of DNA-based optical signal processors is an example of the wide applicability of bacteriorhodopsin and other proteins on which new discoveries in bioelectronics will be based.

Technical Enablers and Barriers

Biomolecular electronics is many years, if not decades, behind computer engineering but is at the forefront of biotechnology. Change, when it occurs, will occur quickly and will result in devices that could be lighter, faster, and possibly cheaper than computer-engineered devices currently used by the Army. The key enablers and barriers to the development of biomolecular hybrid devices are summarized in Box 4-1. The arrows linking enablers with barriers emphasize that an enabler can only be fully implemented if the linked barrier(s) have been addressed.

Two technology enablers may hold the key to genetic engineering and hence the successful development of bioelectronic hybrid devices, including future sensing devices for the Army. Designed combinatorial libraries allow the preparation of *de novo* proteins. By choosing the libraries intelligently, one could generate a small group (fewer than 100) of potential proteins, all of which would have a reasonable probability of yielding enhanced properties. Screening this group would be relatively easy. An alternative would be to use directed evolution, in other words, allow the bacteria to generate random mutations in the protein and provide some method of testing the value of the protein while it is still inside the animal.

Both of these methods would enable a large number of potential mutations to be tested in a short period of time, provided the screening could be done efficiently and accurately. Therein lies the problem. Genetic selection for the desired properties is difficult, and it is unlikely that a global solution can span a large range of target systems. Thus,

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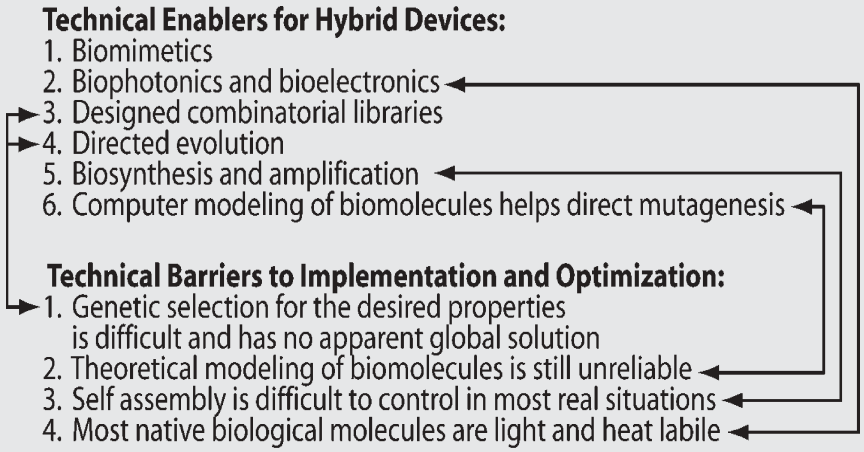
BOX 4-1
Technical Enablers and Barriers to the Development of Biomolecular Hybrid Devices



further research in both combinatorial libraries and directed evolution will be essential to the future development of bioelectronics and biosensors.

BIOCOMPUTING

Biocomputation is a hybrid field that combines computer science and biology (1) to build computational models of real biological systems, using the tools and concepts of information science, so that biological systems can be seen from a different theoretical perspective and/or (2) to use biological systems or processes as metaphor, inspiration, or enabler for the development of new computing technologies and new areas of computer science. For purposes of this report, the term *biocomputing* does not include using computers for analysis or data management in biology, which is called bioinformatics (data handling) or computational biology (simulations). Biocomputation focuses on the hybrid field of computer science and biology, including the computational properties of cells (e.g., genetic regulatory circuits), DNA computation, DNA self-assembly, cellular and DNA logic gates, computer immune systems for combating computer viruses, artificial life (Alife), artificial neural nets, and genetic and evolutionary algorithms. The latter three areas were among the first fields developed combining information science and biology; in fact, artificial neural nets have been in existence since the 1940s.

Biocomputing is an important emerging discipline. Not only is it a rich field for scientific inquiry, but it may also give rise to a wide range of novel engineering technologies with considerable industrial potential. An understanding of how genetic information determines an organism's structure and function may provide insights into the construction of new materials and structures on a molecular scale; conversely, concepts from computer science may shed new light on how the genetic code evolved, and even on the principles of life itself.

For example, certain biocomputing practices may offer a viable alternative to semiconductor-based computing, which might circumvent the problems anticipated by Moore's law in the next few years. (Moore's law, named for the founder of Intel, predicts that the density of components possible on a computer chip will approximately double every 18 months.)

Biological Models

Certain biological organisms, such as cells, can serve as models for sensors or digital logic gates. Computer science concepts are helping molecular biologists analyze and simulate genetic regulatory networks by understanding how cells process information. By viewing biological systems as information-processing units with regulatory logic and circuits, the mechanisms, design principles, and dynamic behavior of regulatory networks in cells can be better understood. A related area is the development of cellular logic gates, whereby a cellular "inverter" can be made based on proteins that either suppress or activate the production of other proteins, the suppression/activation mechanism acting as an on/off electrical switch.

Artificial neural nets, another example, are computer algorithms that mimic the way neurons in the brain process

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information via the generation and transmission of electrical signals. They are used in a kind of iterative methodology, whereby real data are used to train the artificial neural net to recognize certain patterns (e.g., Ford Motor Company has explored using neural nets to recognize engine misfires). Evolutionary and genetic algorithms are computer programs based on the concept of simulating evolution via the processes of natural selection, mutation, and reproduction.

Artificial life, or Alife, is a new discipline that studies "natural" life by attempting to recreate biological phenomena from scratch using computers and other artificial media. Just as synthetic chemistry enables scientists to create chemicals not found in nature, the goal of Alife research is to create biological phenomena in nonliving media.

These are only a few examples of the extensive potential for scientific and technological innovation arising from the confluence of biology and computer science. Biological processes are likely to continue to inspire versatile and useful applications.

DNA Computing

Even though biologically inspired computing technologies may only prove to be useful for very specialized problems, their potential is still impressive. For example, compared to conventional computers, DNA used as a computing medium may prove to be a billion times more energy-efficient and to have a trillion times more data-storage capacity. (DNA stores information at a density of about 1 bit/nm^3, about a trillion times as efficient as videotape.)

DNA computing is also massively parallel. Researchers are currently trying to exploit these properties for several purposes, including solving NP-complete problems (mathematical problems whose answers cannot be checked in computer running time bounded by a polynomial solution), searching large databases, solving problems that require vast amounts of memory, and encrypting data.

Originated by Leonard Adleman at the University of Southern California in 1994, DNA computation makes use of the encoding properties of strands of DNA subunits to compute the solution to problems. This innovative method of problem solving may be useful for problems that would be intractable by traditional computing methods. Adleman was the first to use DNA encoding and storage properties and manipulate single strands so that they could link up in ways that represent the solution to a problem (Adleman, 1994).

DNA consists of two long chains of alternating phosphate and deoxyribose units twisted into a double helix and joined by hydrogen bonds between two pairs of nucleotides, adenine and thymine (A and T) or cytosine and guanine (C and G). In living organisms, each base pair bonds with its complement—A to T and C to G—in a sequence that determines the organism's hereditary characteristics. To use DNA in a computation, one must first puzzle out which sequences reacting in which ways accurately replicate the algorithm in question and then custom make the single strands of DNA with the desired sequences, known as an oligonucleotides.

Typically, a DNA-based computation is arranged as a series of test tubes filled with water and up to 1,020 strands of DNA. Each test tube is created from earlier ones by one of several operations, such as separating the strands by length, pouring one test tube into another, extracting strands with a given pattern, heating or cooling, or using enzymes to splice the DNA. The series of test tubes form a single-instruction-multiple-data computation performed in parallel on the DNA. In 1994, Adleman first successfully tested the theory of DNA computing on the directed Hamiltonian path, or "traveling salesman," problem. The challenge is to figure out a single route that would take a "salesman" to multiple cities from a given starting point to a given end point, passing through each city once and only once. Using recombinant DNA laboratory methods, Adleman was able to extract the correct answer to the traveling salesman problem out of the many random paths represented by the DNA.

Related problems amenable to DNA computing solutions are optimal shop scheduling and the longest path in a graph (Adleman, 1994; Gifford, 1994). Other candidates for DNA computing are cryptography, problems from computer-aided design (e.g., such as checking out the correctness of circuits or protocols), parallel searches, and factoring. Scientists have investigated using DNA computation for encryption and to self-assemble lattice structures on the nanometer scale, which could ultimately be used for nanomanufacturing processes. Other scientists have devised methods of computing with DNA on substrates. DNA has also been used as a data-storage medium, for which associative search methods are being developed for retrieving the encoded information. DNA computing might be used to integrate a complex array of intelligence data into a strategic plan (Forbes, 2000).

DNA computing is not a general-purpose type of computing and is, therefore, inherently limited to the types of problems discussed above. Other weaknesses may also limit the value of DNA computing. For example, individual operations are slow, and set-up time and materials costs associated with complex problems can be considerable. Complex problems may also prove to be volumetrically limited. It takes 0.5 grams of DNA to make 2^{56} strands of 1,000-unit length. To make 2^{70} strands of the same length requires 8 kilograms of DNA.

Initial forms of DNA computers are error prone. Nature has long relied on errors in the DNA replication process for evolutionary advancement. Sometimes these errors enhance the organism, but in most cases they damage an organism and lead to its early demise. Errors are sufficiently common that evolution has spawned an entire protein-based process to monitor and correct errors that occur during replication. Current DNA computing does not take this tendency into account, which could lead to erroneous results.

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In summary, early research in DNA computing shows it to be inflexible with respect to application domain and expensive in terms of logistic demands for space and materials. Newer applications, based on such things as programmed mutagenesis or using DNA as scaffolding, may lead to more adaptable DNA computing algorithms. Overall, however, the possible benefits appear to be outweighed by likely problems in field applications. The Army should continue to monitor basic research in this field, however, because new methodologies might yield new paradigms that permit faster, less expensive, or more general applications.

KEY RECOMMENDATIONS

As the Army becomes increasingly dependent on semiconductor electronics it becomes ever more vulnerable to the effects of radiation and extreme electromagnetic pulses associated with detonations of nuclear or other high-radiation weapons. Unprotected electronic components, which are critical to the Army's command and control, communications, computers, intelligence, sensors, and reconnaissance (C4ISR) capabilities, are especially vulnerable. Furthermore, there is a limit to how well semiconductor electronics can be protected from electromagnetic radiation because the two key schemes used to protect them (high redundancy and Faraday isolation) add significant weight and increase power consumption.

The development of biomolecular hybrid components may reduce this vulnerability to radiation extremes. The extent to which bioelectronic components are inherently insensitive to radiation has not yet been fully explored. Because none of the mechanisms responsible for electromagnetic-induced catastrophic failures of semiconductor devices would be active in biomolecular electronic devices, logic would suggest that these devices would exhibit high tolerance. Clearly, this biotechnology is important to the Army, but has limited nonmilitary applications. Therefore research funding will have to be supplied by the Army.

Proteins are the essential components of protein-based materials and devices that will result from advances in molecular electronics. The value of these devices to the Army will increase significantly, as the proteins are improved and optimized for specific bioelectronic and biosensor applications. Protein developments could also be valuable in the development of new threat agents by potential adversaries; thus, they are potentially important to defense against chemical and biological agents. For these reasons the Army should closely monitor commercial and academic developments to optimize protein characteristics through discovery and genetic engineering.

Protein-based devices that have already demonstrated some potential will require support from the Army to ensure future developments that meet Army needs. The Army should support the development of protein-based data-storage and associative-memory devices, which have been identified as being well suited to meeting military requirements for rugged data-storage media and data-fusion applications, respectively. Army sponsorship will be necessary to ensure that these devices are optimized for use in the field.

The extent to which biomolecular hybrid components are inherently insensitive to radiation extremes has not been fully explored. None of the mechanisms responsible for electromagnetic-induced catastrophic failure within semiconductor devices would be active in biomolecular electronic devices. The vulnerability of C4ISR systems is of critical concern, and the Army should support research to determine the extent to which bioelectronic components are resistant to radiation-induced failure.

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5

In Search of New Materials

Biomaterials and biologically inspired materials at the intersections of biology, medicine, nanoscience, and biomimetics have the potential to revolutionize the design and fabric of future Army systems. "Materials" form the basis for practically all biotechnologies discussed in this report, but this chapter discusses materials to meet the Army's needs for protection and repair, which can be divided into two categories depending on whether the application is *in vivo* (e.g., wound healing) or external (e.g., clothing, camouflage, armor).

Materials for *in vivo* use, often referred to as biomaterials, must satisfy biocompatibility requirements, such as those required by the Food and Drug Administration (FDA) in the course of its approval for safety and effectiveness for intended use. Materials for external use need not be biologically compatible, but biology is still the inspiration for their design and fabrication. Synthetic materials, soft or hard, molecular scale or macroscale, that are inspired by biology are called *bioinspired* materials (Aksay and Weiner, 1998).

Hybrid materials, which are neither bioinspired nor biologically produced, are engineered materials that have one component that is a macromolecule (e.g., DNA, a lipid, a polysaccharide, a polymer, or a protein). Hybrid materials include functional and structural materials, such as may be used in biosensors, and engineered hard tissues, such as bone and enamel, many of which are the result of interdisciplinary developments in biomimetics and nanoscience.

This chapter describes research and development in the private sector that will be important to future Army applications. All in all, molecular-level research in materials of all kinds will be essential to the realization of most of the opportunities highlighted in this chapter (and in this report as a whole).

BIOMATERIALS FOR *IN VIVO* USE

A primary goal for *in vivo* applications is to produce self-replicating biomaterials for wound healing that can be used to heal wounds and repair bones. Materials of choice include bioactive/resorbable/degradable polymer scaffolds, bone grafts, and other materials that might assist in the regeneration of human tissues. If regeneration is not an option, tissue engineering may provide cell-integrating artificial materials (or devices) to replace or enhance the functions of human organs.

Wound Healing

Numerous, often redundant, physiologic mechanisms have evolved for the repair or regeneration of injured tissues (see Box 5-1). During repair, injured tissues are replaced with dense, organized, connective tissues (scar tissue) that may or may not return total function. During regeneration, injured tissues are replaced with structurally and functionally normal tissues. The regeneration of injured tissues is common during fetal or newborn development; scar tissue healing is more common in adults. When the reason for the change is understood, the next stage of major advancements in the treatment of defective, injured, or missing tissues and organs is likely to follow.

A variety of biomaterials are used to treat wounds, including synthetic materials, nonsynthetic materials,

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BOX 5-1
Overview of Wound Healing

Regardless of the injury mechanism (e.g., infection, trauma, surgery), several critical factors are common to the wound-healing process. These factors include an adequate blood supply to the healing tissue, resolution of associated infections, infiltration of the wound site by inflammatory cells followed by mesenchymal cells, and finally the deposition of neoconnective tissues and epithelial tissues.

An adequate blood supply to injured tissue has long been recognized as vital to healing. Cupping, the practice of applying a cup heated by a flame over the site of injury, was used for centuries as a means of ensuring blood flow to topical wounds. The flame consumed oxygen, creating a vacuum and thus drawing blood in the underlying tissue toward the surface. Today, angiogenic factors are delivered to the sites of injury to stimulate the formation of new blood vessels at appropriate times and locations.

Hyperbaric oxygen chambers have been devised to increase oxygen concentration for cells at the site of injury and thereby increase their viability and rate of proliferation. Acupuncture, massage therapy, and a variety of poultices have been used to create the optimal wound-healing environment, especially for recalcitrant, nonhealing wounds.

Wounds have been divided according to their severity, depth, and chronicity. Each category has its own standards of care. However, the principles of cleanliness, wound covering, tissue apposition, and protection from physical trauma while healing tissues return to their normal physiologic state are applicable to all wounds.

A variety of coverings are used for acute and chronic wounds. Dressings range from totally occlusive dressings, which do not allow fluid (and allow little gas) to pass from the underlying wound to the outer environment, to partially occlusive or nonocclusive dressings, which remain permeable to both fluids and gases. Dressings may or may not be carriers of antiseptic or antibiotic compounds. In general, wound coverings for acute traumatic wounds are adequate for treating infections and protecting wounds from further injury. However, there is a pressing need for wound coverings that simultaneously provide, protect, and deliver a stimulus for wound healing. Stimulation for healing is especially important for large defects when "space" must be "filled." In natural healing, large pockets at sites of injury are filled with fluid (usually plasma and/or blood) by the host, which subsequently creates a barrier to rapid healing. Therefore, dressings that not only cover the wound, but also stimulate the formation of new blood vessels and the deposition of connective tissue would greatly improve wound care.

There is an inevitable gap between *in vitro* phenomena, which occur under carefully controlled conditions, such as ideal concentrations of growth factors that have predictable effects on selected cell lines, and practical situations, which involve the complex of mammalian systems and a plethora of different growth factors (both stimulatory and inhibitory) in environments complicated by infection, tissue necrosis, and external extremes. Although several angiogenetic growth factors have been identified, controlling their activity *in vivo* remains elusive, probably because of a lack of understanding of the extracellular milieu of the growth factors *in vivo*. Although the sources of growth factors have been identified (e.g., endothelial cells, macrophages, fibroblasts), the mechanisms that stimulate their controlled release and the three-dimensional ultrastructure in which they naturally reside are not well understood. Therefore, it should not be surprising that growth factors attached to synthetic polymers such as polylactic acid and Marlex mesh are not particularly effective. Similarly, bioartificial membranes comprised of selected molecules, such as hyaluronic acid or purified Type I collagen laced with a variety of growth factors, usually fail to produce the desired effect when applied in clinical situations.

Source: Badylak, 2000.

resorbable materials, nonresorbable materials, and materials used as carriers for biologic agents, such as growth factors, antibiotics, and procoagulants. Each biomaterial is well suited for certain uses. However, none provides an optimal environment for wound healing. Most available biomaterials are used as temporary wound coverings that are later removed to allow the body to heal itself. Future generations of biomaterials for wound care may not only protect acute wounds, but may also set the stage for accelerated healing. Biomaterials that provide a microenvironment suitable for and conducive to physiologic phenomena, such as angiogenesis and cellular proliferation and differentiation, should be targeted by the Army for development.

Biologically produced materials will offer inherent advantages over synthetic materials for wound healing, because they may be self-healing and self-replicating. The development and use of a range of cellular growth factors, as well as stem cells, to facilitate wound healing will be important areas of focus. Engineering of human tissue, both soft and hard, will be a cell-based process that is based on an understanding of how cellular systems organize and communicate at interfaces.

The next major advances in wound healing are likely to be in biomaterials that provide an appropriate environment for immediate cell attachment, proliferation, and differentiation. These advanced biomaterials will provide an

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environment for the growth of new blood vessels, mesenchymal cell infiltration and subsequent deposition of a neomatrix, and the attachment or proliferation of cells that provide protective coverings (e.g., keratinocytes in the skin, mucosal epithelial cells at other body sites).

Identifying biomaterials or biologic agents that promote organized cell proliferation and differentiation will be an active area of research. However, these agents will have to work in concert with an optimal environment (i.e., a biomaterial) to make more than an incremental improvement because they invariably work in the complex environment of the extracellular matrix ultrastructure (Badylak, 2000).

Finally, the next generation of biomaterials for the treatment of wounds will almost undoubtedly have to be resorbable. Synthetic materials, no matter how biocompatible, function as foreign bodies in the mammalian system, which reacts to them in a variety of ways, including encapsulation, infiltration with connective tissues, and/or nonphysiologic cellular response.

Fifty-five percent of battlefield mortalities are the result of excessive bleeding; therefore, soldiers must receive immediate care to survive on highly lethal battlefields (Jette, 2000). Biological materials are now known that have excellent adhesive properties and can help stop bleeding. These include adhesives from barnacles and blood fractions, such as fibrin. Biosealants with excellent adhesive properties might be developed (e.g., by modifying protein biopolymers), and individual soldiers might carry them in their backpacks. The biosealant would act as a "super glue" to stop bleeding and hemorrhaging until the injured soldier could be evacuated to a more permanent treatment setting. However, Army-sponsored research and development will probably be necessary because the private sector is not likely to pursue the specific technologies that would meet soldier needs.

Tissue Engineering

Tissue engineering, a relatively new field, involves combining synthetic materials or structures with living cells. Several research areas in tissue engineering are described below: cartilage repair and replacement; self-replicating systems; stem cells; synthetic biomaterials; bridges between electronics and the nervous system; and portable, artificial, assisting devices.

Cartilage Repair and Replacement

One example of tissue engineering would be the repair of cartilage damage. Although this might not be a lifesaving procedure, it could substantially reduce recovery time from wounds. The technology for repairing cartilage has advanced dramatically. *In vitro* formations of cartilage cells (chondrocytes) have been reported (Binette et al., 1998, and Guo et al., 1989). Commercial development of *in vivo* injections of cartilage cells for regeneration and repair is also quite advanced, and a device produced by Genzyme Tissue Repair, a company in Cambridge, Massachusetts, has received FDA approval for injection into humans. At this point, the Army has an excellent opportunity to establish partnerships with commercial developers to ensure that battlefield requirements are met.

Self-Replicating Systems

Self-replicating systems *in vitro* or *in vivo* could benefit soldiers in the field or during recovery from wounds. Advances in *in vitro* biomaterials have reached the commercialization stage. The cultivation of human fibroblast cells in culture has been demonstrated, and artificially produced human skins have successfully undergone clinical trials by several companies, including Advanced Tissue Sciences, San Diego, California, and Organogenesis, Canton, Massachusetts (Gentzkow et al., 1996).

The *in vitro* cultivation of human skin could save the lives of soldiers with severe burns or other injuries that require skin replacement. However, with present technology artificially produced skin requires stringent low-temperature storage, which would be extremely difficult to provide under battlefield conditions. Therefore, alternative methods for long-term storage will have to be developed, probably through research supported by the Army. It may be possible for a mobile unit to cultivate skin *in vitro* at mass casualty sites. However, the development of supporting technologies will require significant collaboration between the Army and the private sector.

Another example of a self-replicating system would be the regeneration of bones. Scientists have shown that bone morphogenesis is controlled at the genetic level, and a bone morphogenesis factor has been isolated that can regenerate bone both *in vivo* and *in vitro*. This technology is already at an advanced stage and will someday be used to regenerate bone damaged on the battlefield. A new drug that stimulates the healing of bone fractures, called OP-1, is awaiting FDA approval. However, the relatively long time required for the remediation of medium- to large-sized bone defects, especially with collateral repair of skin damage, would limit its applicability in battlefield situations.

Revascularization might be accelerated by the growth of new blood vessels (angiogenesis) in parts of the body where blood flow has been interrupted by battlefield trauma. This approach, which would require preliminary and concurrent homeostasis to avoid irreversible bleeding, could be implemented by the local administration of angiogenesis stimulants, such as vascular endothelial growth factors, or by the implantation of cells that secrete angiogenetic proteins.

Some research is focused on culturing bone marrow cells, which manufactures blood, and it should be possible to create bioreactors in which cultured bone marrow cells can be used to produce blood. In the future, bone marrow cells could be extracted from a soldier when needed, multiplied in culture, and then returned to the patient. This technology

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might also be used to treat bone marrow cells damaged by radiation.

Stem Cells

Stem cells can replace cells that die or are damaged. Some stem cells are self-renewing and can form cells like themselves; others produce specific differentiated cells. Stem cells that produce cells like themselves (e.g., sperm cells) are called *omnipotent* cells. Stem cells that give rise to various types of cells in the nervous system or in the blood or immune system are called *multipotent cells* (e.g., neural or hemopoietic cells). *Immortalized pluripotent* stem cells have both characteristics; they can proliferate and form more cells like themselves, and they can differentiate into other types of cells when culture conditions are modified.

Pluripotent cells cannot make an embryo on their own, however. Therefore, they are not *totipotent* cells, which can give rise to unlike cells and can develop into or generate a new organism or part. Current research is focused principally on pluripotent cell lines derived from mouse embryonic germ cells (other species were found to be extremely difficult to work with). In 1998, researchers at the University of Wisconsin derived human pluripotent stem cells from early human embryos either from patients undergoing treatment for infertility or from aborted fetal material. Since then, intense research has been conducted to induce differentiation in pluripotential stem cells by regulating biomolecules or by other genetic methods. Ultimately, tissues, or even organs, could be supplied to surgeons for transplantation. Possibilities include cardiac muscle cells, pancreatic islet cells for treating diabetes, liver cells for treating hepatitis, and neural cells for treating Parkinson's disease or Alzheimer's disease. Many ethical questions have been raised about research on and the applications of stem cell technologies, because embryonic stem cells are currently derived from early human embryos (blastocysts). Stem cells derived from human umbilical cord blood is a promising alternative; stem cells from human adults would be the least controversial.

Animal research is likely to move ahead more quickly because transferring nuclei (i.e, combining embryonic cells with unfertilized eggs) to obtain an animal with a specified trait is already widely practiced. The genotype of the cultured cells can be changed through genetic targeting to remove or replace genes. This could lead to animals with disease resistance, for example, or animals capable of producing valuable protein biopharmaceuticals in their milk. Cloning of animals has been successful for sheep, cows, goats, pigs, mice, and other animals (see Figure 5-1). Extrapolating the trend to human body parts, however, is unlikely in the near future because many clones do not make it to full term and because the low success rate (less than 2 percent) translates into very high costs.

The ultimate application of stem cell research would be treating human patients directly with tissue implants, assuming that rejection could be overcome. According to a report by Clarke et al. (2000), neural stem cells from an adult mouse brain were shown to be capable of contributing to the formation of chimeric chick and mouse embryos (see Figure 5-2). This would suggest that adult animal cells are able to revert to behavior associated with stem cells.

FIGURE 5-1 Dolly the sheep.
Source: Reprinted with permission Roslin Institute, Midlothian, United Kingdom.

Perhaps by 2025, somatic cells obtained directly from patients could be used for transfer of nuclei. Pluripotent stem cells derived from somatic cells would be antigenically identical to existing cells in the patient and, therefore, at least in theory, would not be rejected. Imagine transplants that did not require antirejection drugs or skin cells that could be rapidly regenerated to treat burn patients. However exciting the prospects, the Army should be wary of promises of immediate success but should continue to monitor technological, social, and bioethical developments. When fundamental knowledge of cell biology is improved, the possibilities can be mapped more realistically (Clarke et al., 2000; McLaren, 2000; Pennisi and Vogel, 2000).

Despite almost 30 years of active research in the field, tissue engineering to transplant islet cells destroyed by diabetes has not become a clinical reality. Low success rates in clinical trials have been compounded by the necessity of giving transplant recipients antirejection drugs that weaken the immune system and open the door to opportunistic infections or posttransplant malignant diseases. New hope has been generated by the development of a method of harvesting and injecting islet cells into the circulation in a manner that allows them to lodge stably in the liver. With this methodolology, pioneered by researchers in Edmonton, Alberta, Canada, it has been possible to render all 15 patients in a clinical study completely insulin-independent, although they are still on an antirejection regime.

Hopes have also been raised by animal experiments show-

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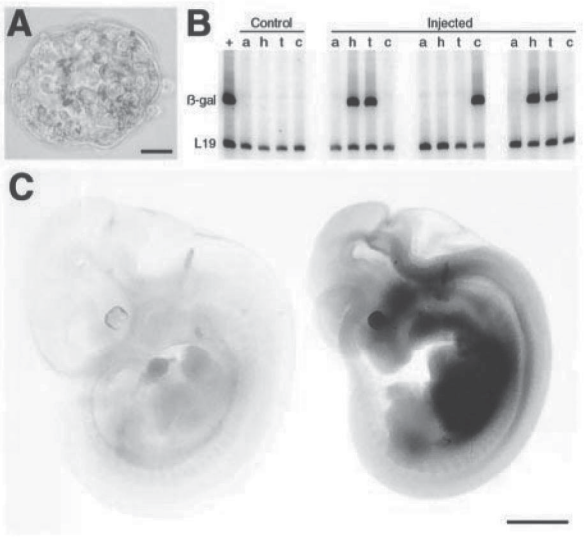

FIGURE 5-2 Generation of chimeric mouse embryos. (A) X-Gal staining of a blastocyst developed *in vitro* from a morula aggregation experiment shows neural stem cell-derived cells in the inner cell mass. (B) RT-PCR detection of B-Gal mRNA in ROSA26-derived adult neural stem cells (+), amnion (a), head region (h), trunk (t), and caudal part (c) of a wild-type embryo (control) and embryos generated by blastocyst injection of ROSA26 neural stem cells (injected embryos other than shown in C). Primers for L19 gene were included in all reactions as an internal control. (C) X-Gal staining of an embryonic day 11 wild-type embryo (left) and a mouse embryo generated from a blastocyst into which adult neural stem cells were injected (right). Some endogenous staining is seen in the area of otic vesicle in the wild-type embryo. Bars in lower right corners denote lengths: 20 μm (A) and 1 mm (C).

Source: Reprinted with permission from Clarke et al., 2000. Copyright 2000, American Association for the Advancement of Science.

ing that implantable silicon microchips may effectively shield transplanted cells from rejection, thus opening the way for a new generation of implantable therapeutic cell bioreactors. The Army should monitor stem cell research for possible applications to trauma, the repair of wounds, and some types of cellular or organ transplants.

Synthetic Biomaterials

Another example of tissue engineering is biomaterials or compatible materials constructed of human parts, (e.g., heart valves and arteries). Development and use of these artificial devices will require a thorough understanding of biological functions, toxicity, and other factors. An understanding of surface chemistry and its implications for biological function will be essential to ensure biocompatibility. Recent advances in tailoring and characterizing surfaces at the molecular level are providing insights into how cells and tissues organize at interfaces. Continued development will lead to new methods of integrating biological and synthetic components for the generation of hybrid devices that may facilitate *in vivo* communications between biological events and electronic devices. Significant research in this area is already under way, and the Army should monitor developments to determine if they can be adapted to soldier applications.

One area of special interest is the transplantation of cell lines to provide an implantable biochemical factory for the production of life-sustaining or performance-enhancing molecules. Among the former are blood-clotting factors and insulin; the latter could include proteins that influence responses to fear, sleep deprivation, and fatigue. The synthetic component of these constructs is in the immunoisolating external shield, which is designed to protect them against the rejection response. Private industry is investing heavily in immunoisolating technologies (e.g., Neocrine, Inc.) thus providing the Army with opportunities for leveraging private investments.

Bridges Between Electronics and the Nervous System

For more than three decades, researchers have been exploring the coupling of electronics and the nervous system at the cellular level, both as a means of understanding neural functions and as a means of developing prostheses to mitigate, or even "cure," a number of debilitating neural disorders (Agnew and McCreery, 1990). Considerable work is under way to develop retinal and cortical implants to restore vision to the blind, to restore movement to paralyzed limbs via functional neuromuscular stimulation, and to restore hearing to the profoundly deaf. Of these, the hearing prosthesis is the most completely developed; more than 30,000 first-generation devices have been implanted worldwide. Success rates with these implants continue to increase, enabling many patients to function normally in a hearing world. Dramatic results are expected in neural prostheses in the next decade.

As the risks and costs associated with neural implants are reduced, they may be used to increase the visual and hearing acuity of unimpaired individuals to levels well above average. Soldiers possessing these extraordinary faculties would be well suited to gathering intelligence and performing long-range reconnaissance missions. Success for some types of implants will depend on resolving biocompatibility issues. (See section Implants and Biocompatibility in Chapter 7.)

Most current neural systems use distributed arrays of individual wire electrodes (25–50μm in diameter). Future systems with higher levels of capability and more sites are likely to use micromachined electrode arrays (see Figure 5-3) (Najafi et al., 1985). After 30 years of research, three-dimensional arrays can now be formed with as many as 1,024 stimulation or recording sites spaced on 100μm–400μm centers (Bai and Wise, 2000; Rousche and Norman, 1998). With embedded circuitry, electrode sites can be positioned electronically to couple with active neurons (Ji and Wise, 1992),

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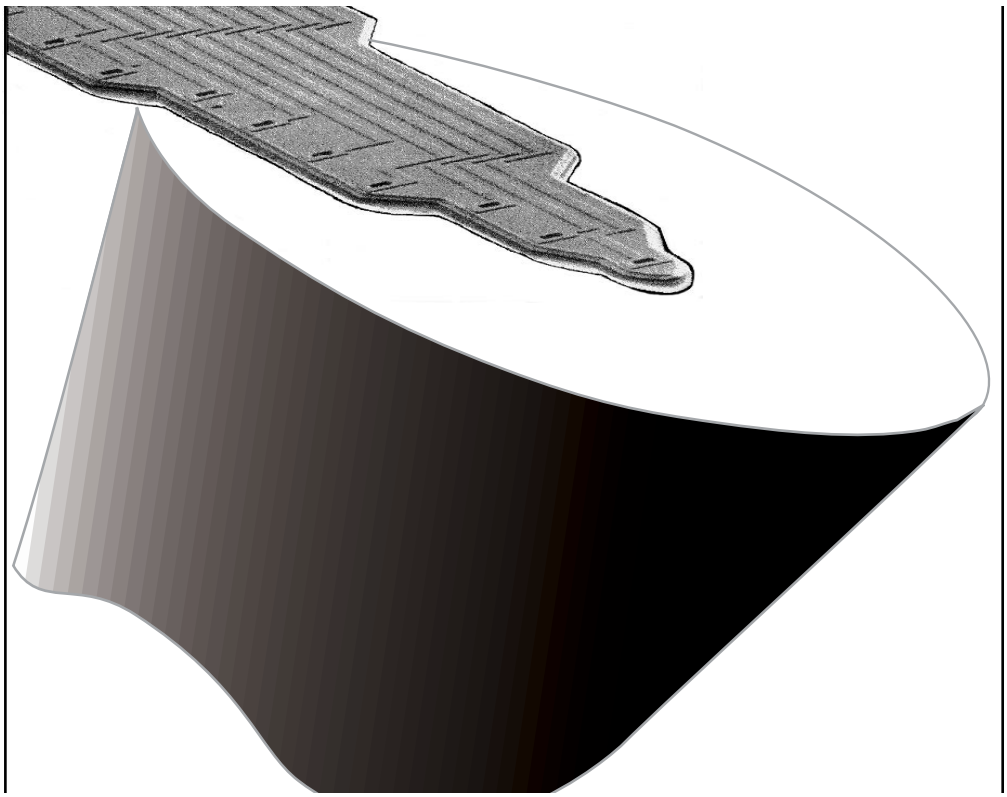

FIGURE 5-3 Scanning electron micrograph of a multisite, micromachined, neural-recording probe. The probe size is compared to a cross section of a strand of hair at the same magnification.

Source: Courtesy Professor Ken Wise, University of Michigan.

and the resulting extracellular signals can be amplified, multiplexed, and passed to external signal processing systems. In the near future, such devices could interface to the outside world over bidirectional telemetry links that will also supply power via radio frequency coupling (Von Arx and Najafi, 1999). These arrays are potentially capable of completely instrumenting blocks of intact neural tissue while displacing less than 1 percent of the tissue volume. The hope is that these arrays will be able to record from neural networks on a very long-term basis without disturbing their normal function and will be able to insert signals into the tissue electronically. Hence, they would form a bridge between the electronic and biological worlds.

Thin-film, micromachined electrode arrays are used worldwide in acute situations (Carter and Houk, 1993), and the technology for completely implantable neuroelectronic microsystems will be available in the next decade. However, before these systems can be routinely used in prostheses, coupling between the electrodes and neuronal circuits must be made stable (Wolpaw et al., 2000). Currently, contact sites are encapsulated by cellular membranes or protein over a period of weeks or months. Although stimulating currents can pass through these layers with little effect, the films shield recording sites from the neural signals disrupting electrode-cell coupling. A variety of solutions to this problem are being explored, including improvements in site structure and site coatings based on genetically engineered polymers designed to inhibit protein adsorption and growth. Microwire electrodes have successfully recorded from neurons *in vivo* for periods of up to two years, permitting a simple robot arm to be controlled by signals from the motor cortex (Chapin et al., 1999; Kennedy and Bakay, 1997).

Research on neuroelectronic interfaces is significantly advancing our understanding of biological neural networks and is expected to lead to considerable progress in the treatment of neural disorders in the next decade. Although several highly sophisticated microneural probes have been developed in the past 20 years, including probes with complex electronic circuitry, significant problems with biocompatibility must still be overcome. In fact, these biocompatibility problems have limited their usefulness and applications. Long-term stability of the materials, rejection by the host cells, and loss of connections with neurons or cells still plague these promising devices. Significant improvements in biocompatibility will be necessary before the potential of these devices can be fully realized.

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Portable, Artificial, Assisting Devices

Imagine a wound received in battle damaging one or more organs vital to survival, e.g., heart, liver, lung. The development of artificial devices for treating such acute damage is in very early stages; for example, a number of artificial devices for replacing liver functions are now being tested in clinical trials. So far, questions of how these devices might be deployed on the battlefield are not being addressed. Once the Army has determined the requirements of soldiers under battlefield conditions, it should be able to capitalize on commercial developments.

Many tissue-engineered regenerative parts will certainly be available by 2025. At the same time, however, high-performance replacement parts for humans made wholly or partly of nonbiological materials may outperform tissue-engineered products. For example, a high-performance hip implant can easily last 40 years or more and may still be commonly prescribed in 2025, even if tissue-engineered products have been developed. Major trauma to the craniofacial structure, common in battlefield wounds, will also probably require non-tissue-engineered replacement products for the foreseeable future.

BIOINSPIRED AND HYBRID MATERIALS

Bioinspired materials and processes use biological principles to create synthetic analogue composites (Sarikaya and Aksay, 1995). Ideally, nanostructured organic and organic/inorganic composites can be designed and fabricated by mimicking the processes, structures, and properties of biological materials.

Although not necessarily bioinspired, many new hybrid materials will result from biomimetics research encompassing material biosynthesis, protein selection, and other applications. Fundamental understanding of material biosynthesis includes small-particle formations (e.g., ceramics, semiconductors, and metal forming in bacteria and algae), thin films (e.g., S-layer bacteria), and shells and structures (e.g., bone, spicules, spines, dental tissues). Research on protein selection has focused on the development of genetic-engineering techniques (e.g., phage display and cell-surface display techniques) for isolation, selection, and purification of proteins for inorganic and (polymeric) surfaces. Finally, other research applications have included use of biomimetic pathways, as well as the design and synthesizing of improved (hybrid) materials.

Protein structure is closely related to protein function, which in turn is closely related to the properties of materials (see Box 5-2). New protein designs may yield materials with extremely useful properties. Materials such as bones, teeth, and shells are simultaneously hard, strong, and tough and have unique hierarchical structural motifs originating at the nanometer scale.

Nanostructured organic/inorganic materials might also fulfill the Army's need for sensor/actuator arrays, optoelectronic devices, and medical materials. The Army is especially interested in the development of lightweight armor and other materials that can withstand the rigors of combat. Desirable materials would not only be used for weapon systems, but also for uniforms, helmets, munitions, electronics, and other critical applications.

Biocomposites

The structures of biological composites are hierarchically organized in discrete levels or scales. Virtually all biocomposite systems have at least one distinct structural feature at the molecular, nanoscopic, microscopic, and macroscopic scale. In hard materials, nature accomplishes this by growing hierarchically structured organic/inorganic composites in which soft materials (e.g., proteins, membranes, and fibers) organized on length scales of 1 nm to100 nm are used as frameworks for the growth of specifically oriented and shaped inorganics (e.g., $CaCO_3$, SiO_2, Fe_3O_4, hydroxyapatite) with small unit cells (~1nm) (Lowenstam and Weiner, 1989; Sarikaya and Aksay, 1992). The high-modulus inorganic phase provides stiffness while the organic phase enhances toughness. Although the principle of hierarchical design has already been applied to synthetic composites (Lakes, 1993), techniques to reduce the smallest level of hierarchy to the submicron scale are still under development. Hierarchy at the nanometer scale has led to materials properties fundamentally different from those expected based on simple rules for mixing the bulk properties of the constituents (Siegel, 1993).

Levels of structural organization are held together by specific interactions between components. For example, the structure of an abalone shell consists of layered plates of $CaCO_3$ (~200nm) held together by a much thinner (<10nm) "mortar" of organic template (Figure 5-4). (Sarikaya and Aksay, 1992, 1995). Whatever the nature of the bonding between levels, adequate adhesion is required for the system's structural integrity. Structurally organized organic surfaces induce growth of specifically oriented, dissimilar constituents catalytically or epitaxially (Sarikaya and Aksay, 1992). The structure of nacre of abalone (mother-of-pearl) is segmented laminate, a hybrid material in which the dimensions of the component phases play an important role in its impact-resistance and compressive properties.

Highly interacting levels are organized into a hierarchical composite system designed to meet a spectrum of complex functional requirements. As composite systems increase in complexity, they function at higher levels of performance (e.g., so-called intelligent materials and adaptive composite systems). A hierarchical biocomposite is more than just a material out of which larger objects can be built. It is a complete structural system in itself, and nested levels of structural hierarchy appear to yield improved dielectric and mechanical properties for particular functions (Sarikaya and Aksay, 1995; Baer et al., 1987).

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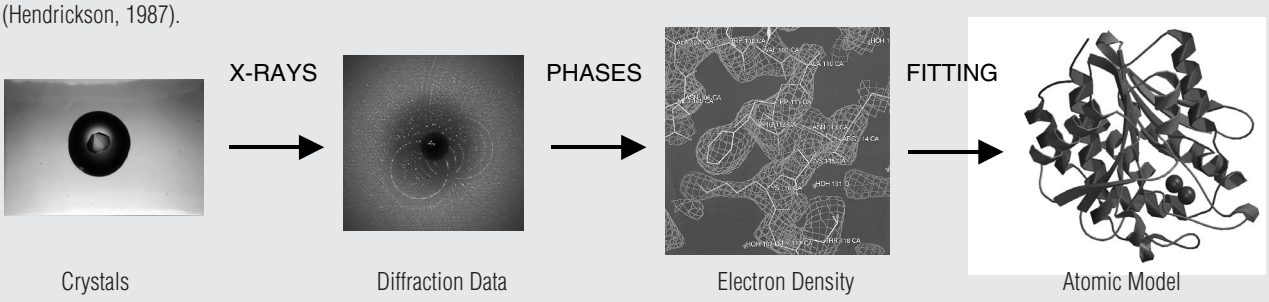
BOX 5-2
Determining the Structure and Function of Proteins

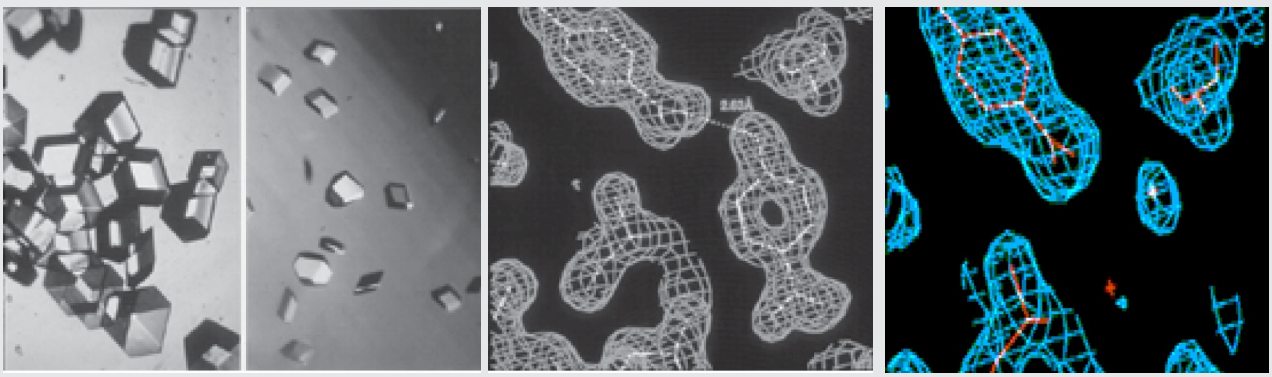
Determining the structures of new proteins is a huge project. The total output of all structural biology laboratories worldwide is currently about 1,000 new structures per year. Most new structures are determined by x-ray crystallography, which involves the basic steps shown in the figure below (Hendrickson, 1987).



High-throughput protein crystallography depends on several new technologies: robotics to automate protein crystallization trials; synchrotron x-ray sources to provide bright, tunable radiation; multiwavelength and purely computational approaches to overcome phase problems; and automated interpretation of electron density maps. The biggest roadblock to progress is the very beginning of the process, namely the expression, purification, and crystallization of the protein, which is both tedious and labor-intensive. Other slow steps are access to the synchrotron radiation needed for data collection at multiple wavelengths on very small crystals and the rapid interpretation of electron density maps of intermediate quality. There are only a handful of synchrotron radiation sources in the world, and these facilities are expensive to operate and in great demand (Hendrickson, 1986).

In microgravity conditions, crystals can be prepared to a size and state of perfection that is otherwise difficult to achieve. Success rates for producing crystals large enough and of sufficient quality for x-ray diffraction analysis are typically less than 40 percent. Microgravity significantly reduces buoyancy-induced convection, thereby providing a more quiescent environment for crystal growth. As a result, crystals grow more slowly and, in about 25 percent of cases, they are of superior diffraction quality than their Earth-grown counterparts. The success rate and magnitude of diffraction enhancement are expected to increase when longer growth times become available via the International Space Station. Experiments on the space shuttle have demonstrated the potential benefits of microgravity. Increased diffraction resolution can often allow researchers to observe structural features that could not be seen using Earth-grown crystals.

Space-grown crystals have provided information about drug-protein interactions that is vital to the design of more effective pharmaceuticals. One example of this involved the determination of the structure of human insulin complexed with a potential drug. With Earth-grown crystals diffracted to lower resolution, it was impossible to tell that two drug compounds were located in the insulin hexamer. Data from crystals grown on STS-60 allowed investigators at the Hauptman Institute to see a second drug complexed in the hexamer structure (see figure below). In addition, the overall resolution was improved considerably (more than 0.5 Å). The Hauptman Institute and a major pharmaceutical company are using this information to design new compounds in an effort to produce a longer-acting insulin formulation (Smith et al., 1996).



The completion of the Human Genome Project, as well as genome projects for dozens of other species, including pathogenic bacteria and viruses, will place a significant demand on and need for crystallographic structure determinations and structure-based drug design. Given the need for methods that might improve the success rates in obtaining high-quality crystals, the microgravity environment could play a significant role in helping to address this important problem.

Source: Delucas, 2000.

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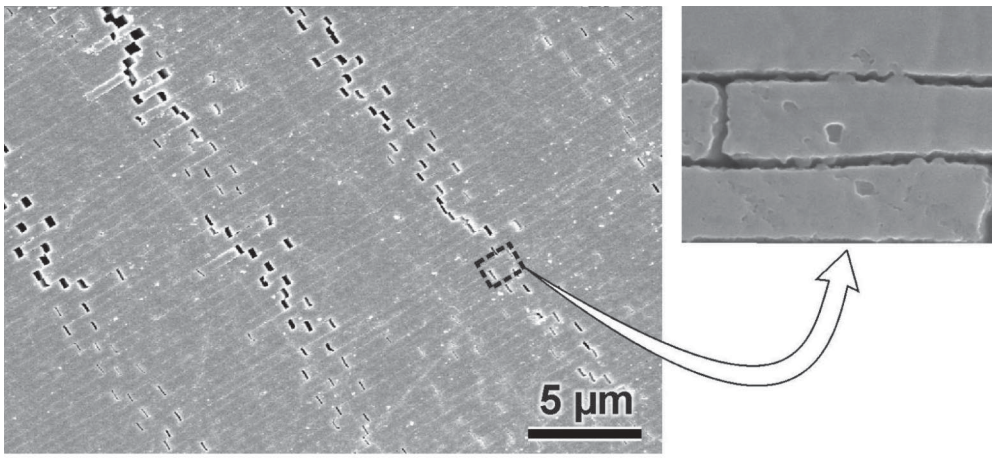

FIGURE 5-4 Structure of an abalone shell.

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Research in hierarchical systems can provide new insight into predictions of materials performance. For example, experimental and modeling studies could be undertaken on materials and systems that have several levels of dimensional hierarchy, each dimension having separate structures and properties that are different from those at other levels. Overall, the system may have novel properties that could not be predicted using conventional approaches but could be predicted based on an understanding of cross-level interactions.

Applications of molecular biology and genetic engineering, including a large number of recombinant proteins for human therapeutics, have been focused mainly in the health care area. Many of these products, including recombinant proteins such as insulin, human growth hormone, factor VIII, erythropoietin, tissue plasminogen activator, and others, have been on the market for a number of years.

Although medical uses of biomaterials have become common, biomaterials created with recombinant DNA technology are not competitive with synthetic materials for general applications because of their high cost. This situation is likely to change in the future as nonmedical uses are found for these materials. The committee believes that biomaterials will become important for meeting the needs of the Army in 2025. Although some of these biomaterials have already been demonstrated to be technically feasible, many challenges and barriers will have to be overcome for them to be realized. A few examples, as well as some of the challenges, are described below.

Silk, a natural biopolymer, is one of the strongest fibers known on a strength-per-unit weight basis. The production of recombinant silk has been demonstrated in bacteria. Private companies (e.g., Protein Polymer Technologies, Inc., San Diego, California; DuPont, Wilmington, Delaware) and the U.S. Army Natick Laboratories have conducted research in this area. Commercial development and production of silk, however, is impractical because of costs. Silk produced by silk worms is much less expensive than recombinant protein production using a bacterial host. Nevertheless, the integration of recombinant technology with materials science and engineering appears to be a natural partnership, and the functionalities of recombinant biopolymer silk may soon surpass those of natural silk.

Professor David Tirrell of the California Institute of Technology has proposed replacing the natural amino acids in native protein polymers using both chemistry and biotechnology to generate materials with unique and added functionalities. With biological production methods, new types of polymers can be produced with well defined, selectable sequences and uniform composition. For example, modified silk may have a significantly higher strength-to-weight ratio than natural silk, increasing its utility for Army applications.

With advances in molecular biology, the sequences of protein-based polymers can be tailored to meet specific needs, and numerous copies could be produced, as desired. The ability to produce highly defined primary structures is well beyond the capability of procedures currently available in polymer chemistry. It is possible, however, that protein-based polymers with highly specific and tailored properties will have molecular weights with singular values rather than a distribution of values, as is the case with synthetic polymers. A serious challenge will be to produce large quantities of these materials so that it becomes economical to use them for specific applications, such as coatings.

Much of the organization inherent to a biological system occurs when one linear strand or chain of an entity folds into a form that features three-dimensional structure and complexity. The preferred folds are coded into the sequence of the chain by side chains with various degrees of association for one another. Although linear structures of almost any composition can now be synthesized, preparing these species

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and having them fold into structures with the desired three-dimensional structure and function can still not be done. Better computational modeling will be necessary to predict the basis of self-assembling, higher order structures.

In addition to advances in fully biologically derived materials, advances in mimicking elements of biological self-assembly will enable more combinations with synthetic materials. Research in molecular biology has produced cottage industries that can supply reagents and tags that bear a variety of biological subunits and the means to attach them to both biological and synthetic systems. These reagents provide a means to introduce characteristics of specific recognition, stimulus responsiveness, and elements of self-assembly into synthetic materials. Based on the use of biological motifs, controls over the thermal, optical, and biochemical responses of materials will soon be available. Future developments will continue to include the newest commercially available precursors, and the committee recommends that the Army inform industry of desired properties for these materials so that new designs reflect Army needs.

A major potential Army application for biomaterials is battlefield armor. For instance, on a strength-to-weight basis, the abalone shell has armor protection capabilities equal to or greater than those of existing materials. When laminated hierarchical structures of biological systems (e.g., the nacre of abalone shell) are mimicked in microlaminated ceramic-metal (Halverson et al., 1989), ceramic-organic composites (Mueller et al., 1997), or organic-organic composites (Baer et al., 1987), significant improvements in the composite mechanical properties have been observed.

Applying a simplified version of this layering to B_4C/Al and other composites results in significant enhancements to their mechanical properties. The B_4C/Al composites are strengthened as a result of residual stresses with nanoscale modulations in the interpenetrating network of the ceramic and metal phases (Kim et al., 1989).

Greenleaf Corporation, in Saegertown, Pennsylvania, has manufactured B_4C/Al tiles for use in armor panels aboard C-130 and C-141 aircraft. The processing of these ceramic/metal and ceramic/organic microlaminates is based on the infiltration of laminated scaffolding (e.g., ceramic) with a liquid (e.g., metal or organic polymer). The laminated composites produced by this method are similar to nacre. Although these accomplishments attest to the value of transferring lessons from biology and mimicking biological structures to create synthetic analogs, the smallest length scale in a complete system is still in the micron range because of the intrinsic limitations of the tape-casting process (Halverson et al., 1989). To capture the most important aspect of structural organization observed in biological systems, methods must be developed to process hierarchical systems with deliberately introduced designs ranging from nanometer to macroscopic dimensions.

In general, research on the biosynthesis of inorganic "biomaterials" has not been as high a priority as research in human health care. Biomaterials could have great benefits for the Army, but it may be up to the Army to demonstrate the technical and economical feasibility of biosynthesis.

Biomineralization: Organic/Inorganic Nanocomposites

The formation of bone is an example of a more general set of processes in which cells act as dynamic, local building blocks that direct the assembly of their extracellular matrix; the matrix, in turn, serves as a template for the growth and function of cells in the formation of tissues (Colognato et al., 1999; Ingber, 1997b; Schwarzbauer, 1999). The formation of bone occurs through the deposition of an inorganic phase, dahllite (carbonated hydroxyapatite), by osteoblasts on an organic template, collagen. This calcified matrix serves as the template for bone formation through the combined action of osteoblasts and osteoclasts (Erlebacher et al., 1995; Mundlos and Olsen, 1997). Bone has a number of important characteristics that could inspire materials design:

- *Complex internal structure that maximizes strength-to-weight.* Bone is a sophisticated composite with several layers of hierarchical structure. In the structural phase, an inorganic material with a polymer (protein) is the matrix. The internal structure is modulated to optimize load bearing. A unique ability that may be related to this internal structure is bone's ability to dissipate energy.
- *Adaptability.* The structure of bone, like many other biological structures, is adaptive. The structure constantly remodels itself to compensate for changes in loads and stresses. On the millimeter scale, bone matrix is deposited in distinct three-dimensional patterns organized to bear mechanical loads locally. All of these mechanosensitive effects are mediated by the cells that inhabit the tissue (Ingber, 1997a).
- *Multifunctional use of internal open space.* Much of the internal structure of the shaft of bone is open. *In vivo*, this space is largely used for microsynthesis, protection, storage of the cells of the haematopoietic system, and fuel (fat) storage. The entire bone is also permeated by a microfluidic system of blood vessels and capillaries that provide for high throughput (input and output) of chemical factors.
- *Integrated sensing.* Bone contains a large number of sensors with complex control architecture to report excessive stress or damage and to control remodeling. Bone tissue, and even bone cells, are especially mechanoresponsive. Altering of stresses induces electric field (piezoelectric effect), drives internal fluid flow, modifies chemical processing rates, and changes cell growth and form.
- *Self-healing.* When a bone is broken, specialized cells (osteoclasts and osteoblasts) dissolve the damaged regions and redeposit new bone. Capillary endothelial

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and smooth muscle cells that mediate the process of angiogenesis and tissue vascularization are also central to self-healing.

The processes involved in bone formation may hold the key to the development of materials with a wide range of exceptional characteristics. Materials could be engineered for Army applications as diverse as lightweight armor vehicles and "smart" clothing for soldiers.

BIOMATERIALS FOR CLOTHING AND CONCEALMENT

Biological systems can also be mimicked for the next generation of soldier camouflage uniforms. One idea is to mimic the mechanical chromatic effects that birds and fruits use. The exquisite color patterns on the feathers of birds are the result of the intricate structural pattern of each feather that enables it to diffract light. This phenomenon, mechanical chromatophores, is also exhibited by some fruits. Another natural phenomenon that might be valuable for camouflage is the biochromatic behavior of some reptiles. The chameleon, for example, can change color and patterns in accordance with the environment. Camouflage with this property would automatically change to blend with the environment, such as snow-covered terrain, desert sand, dense and light vegetation, daylight and darkness. Research in this area would require a commitment by the Army because the private sector is not likely to be very interested in these materials.

Biological means might also be useful for avoiding radar detection. Some biomolecules have long been known to be strong microwave absorbers. For example, bacteriorhodopsin has strong microwave absorptivity (3 GHz to 40 GHz). Scientists are investigating the use of chemically, and possibly genetically, modified bacteriorhodopsin protein as the active medium in microwave-absorbing paint for both tanks and planes. The absorption mechanism appears to be associated with the motion of monovalent and divalent metal cations within channels, e.g., Mg(II), Ca(II). If this theory is correct, proteins could be engineered to have precise microwave absorption bands and then fine tuned for anticipated threats in a given theater of operation. Microtubules, which are also excellent microwave absorbers, may be even better microwave absorbers and more easily fine tuned. Because much of the research in this area is classified, the committee was not able to make recommendations in this area.

The Army is always looking for ways to minimize the detection profiles of its soldiers. Because humans, tanks, and other military structures have a significantly different reflectivity than plants and trees, the enemy can easily identify military targets with inexpensive infrared lasers with wavelength-scanning capability. Even a small fraction of the target is observable because of its distinctive spectral properties. It may be possible to develop paints with terahertz and infrared reflectivity identical to trees or grass, possibly using genetically engineered plant protein as the active medium. Directed evolution could play a major role in the optimization of thermal and photochemical stability that will be needed.

Production of Biomaterials

A major barrier to the production of biomaterials is the lack of economical manufacturing processes. To overcome this barrier, agricultural biotechnology might be used for large-scale production of some new materials (see Renewable Resources in Chapter 6). For example, the protein from soybeans can be refined and sold for only pennies per pound of protein, substantially less than the cost of manufacturing equivalent synthetic polymers. The Army should include the means of production in its evaluation of the potential benefits and costs of biomaterials.

Genetically engineered crops (transgenic crops) could potentially deliver recombinant proteins directly with the food or feed products in which they are found. A recent report by Kusnadi et al. (1998) discussed the production, purification, and characterization of recombinant proteins from *E. coli* and chicken egg white. In this case, β-glucuronidase (GUS) and Avidin were expressed in transgenic corn seed and recovered from the seed. The Avidin made up 5.7 percent of the extractable protein, GUS was 0.7 percent of the extractable protein. Biochemical properties of these proteins were reported to be similar to those of the respective data proteins (derived from *E. coli* or from chicken egg white). The approach has significant potential to generate proteins cost effectively in food crops and might be used on a large scale. Given the potential for this technology to produce enhanced foods as well as specialty (therapeutic) proteins on a large scale, the Army would be well advised to monitor developments in this field.

Manufacturing Trends and Barriers

The production of large-volume, low-cost materials is increasingly based on synthetic chemistry. Although biotechnology may be cheaper and more efficient in some cases, in other cases chemical synthesis of biological materials is sometimes a better option because it can be done using the existing manufacturing infrastructure. Therefore, decisions about manufacturing processes, especially for processes that require synthetic chemistry, should include an evaluation of cost and efficiency. For example, in the production of silk or other protein polymers, biotechnology might be more cost effective than chemical synthesis.

A significant barrier to the development and use of biomaterials is that many will be considered to be, and evaluated as, medical devices by the Food and Drug Administration. Meeting FDA requirements could significantly add to development time.

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KEY RECOMMENDATIONS

The Army can best use its resources by funding basic research that addresses major impediments to the use of biomaterials, rather than for specific developments. For example, advances in understanding the effects of hierarchical structure and molecular properties on integration with biological interfaces will contribute to accelerated developments of implantable sensors, to the robustness of prosthetic devices, and to the manufacture of ultrastrong, resilient, lightweight materials for uniforms, armor, and protective gear. This research in biological interfaces will also benefit biomedical applications for advanced biomaterials for use in the treatment of wounds and trauma.

Exploiting wound-healing applications of biotechnology is of paramount importance for improving the survivability of soldiers in battle. The Army should support research and developments in wound healing in collaboration with the commercial sector to ensure that areas pertinent to battlefield conditions and postbattlefield recovery processes are addressed. Near-term projects should focus on technological barriers to the manufacture and storage of self-replicating systems, such as those used to construct human skin; the results can then be adapted for battlefield conditions. The Army should also take the lead in developing treatments for reducing the incidence of shock resulting from wound trauma; this should be a high priority for research.

Assuming that societal objections can be surmounted, research on the use of stem cells to advance developments in tissue engineering should be supported. The Army should monitor global developments in stem cell research, particularly on human adult stem cells.

The Army's use of biochips and implantable sensors will require that these technologies be stabilized on a material substructure. For this reason, development of biocompatible materials with the properties necessary for the substructure is imperative. This area of research is especially important because the surface is the only part of the material that will interact with a biological milieu. Future engineering developments will depend heavily on reliable, robust, compatible surfaces.

Biological systems, which are models of hierarchical design with multiple functions, could be considered paradigms for the development of synthetic biomaterials. Methods of capturing the structural organization of biological systems have not been developed. The Army should support research on methods of processing hierarchical systems with deliberately introduced designs, ranging from nanometer to macroscopic dimensions, to accelerate advances in tissues in treating wounds, protective clothing, and other battlefield materials.

Such developments could also address a persistent concern expressed in briefings to the committee by the Army about reducing the 92.6-lb weight that soldiers must bear as their combat equipment load (Jette, 2000; Kern, 2000). Clothing contributes to this weight burden, and biotechnology can be used to reduce its weight and increase its utility for protection and camouflage in combat.

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6

Reducing Logistics Requirements

The Army continues to be interested in increasing the "tooth-to-tail" ratio, that is, increasing combat effectiveness and reducing logistics support requirements. Reductions in the logistics burden are especially important at the level of the individual soldier in the field. This chapter discusses trends and developments in biotechnology that could reduce logistics requirements.

Logistics is at the heart of military operations and is a significant factor in success or failure in battle. A major aspect of logistics is providing adequate quantities of fuel, ammunition, food, water, and other consumables to support an operation. Logistics also includes transporting soldiers and equipment, repairing battlefield systems, and providing medical services. *Reducing the Logistics Burden for the Army After Next*, an NRC report published in 1997, recommended that the Army focus on technologies that contribute to reductions in the weight and volume of systems and materials. The same study suggested that reductions could also be achieved by increasing combat effectiveness and that lightening the load soldiers must carry would, in fact, do both. Even a single improvement, such as simplifying a system, changing a process, even altering the dimensions of a cargo bed, can ripple into reductions and savings of major significance.

The biotechnological evolution of sensor technologies discussed in Chapter 3 could enable the Army to chart a course toward the miniaturization of multifunctional systems, such as the laboratory-on-a-chip, which would reduce the logistic burden. Similarly, agricultural biotechnology for creating edible, digestible, nourishing food from raw materials that might be foraged on the battlefield would reduce, or even eliminate, the need to transport food to foreign ports. Water generation and purification using locally supplies is equally important. Finding ways to satisfy energy needs locally would reduce logistics requirements for fuel, as well as help reduce the numbers of batteries needed to power soldier-portable electronics.

The Army currently is engaged in a transformation process to make its organizations and systems capable of responding more rapidly to contingencies anywhere in the world with smaller, lighter forces (Gourley, 2000). The trend toward smaller and lighter underscores the importance of soldiers being able to operate independently and in small units with minimal direct logistics support.

The particular applications that can make a difference in logistics may depend on geography. Sending seeds to Siberia may be worthless, but putting nutrients into candy bars could elevate body temperature. Coatings for the skin could provide insulation from the cold, and "gray-day" photovoltaics could harvest solar energy when there is no sun.

Army priorities for taking advantage of biotechnology will depend on the Army's interpretation of its role in the future. In general, however, anything smaller and lighter will reduce the logistic burden, will further the Army's transformation, and will be in keeping with trends and developments in biotechnology, such as small-scale biological devices, functional foods, biological photovoltaics, and renewable resources.

MINIATURIZATION OF BIOLOGICAL DEVICES

The miniaturization of systems and components would reduce logistics support requirements by making things smaller, lighter, and more portable and by reducing requirements for fuel and power. Small-scale systems may also be easier to repair or replace. The incorporation of biological components into MEMS (bio-MEMS) will help to make nanoscale technologies feasible. Indeed, miniaturization will not only have an impact on Army logistics, but will also make many battlefield concepts that depend upon miniature devices feasible. The development of bio-MEMS is being driven by the commercial market for sensor instrumentation and is likely to lead to near-term breakthroughs in design and manufacturing.

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This section discusses trends toward miniaturization via MEMS and microfluidics to produce systems that are more supportable in the field (i.e., smaller, lighter, faster, and more power-efficient). These trends will have a significant effect on the development of biosensors and biochips. The discussion does not include basic evolutionary changes in microtechnologies that are expected to occur as a matter of course and some systems-engineering trends. For example, incremental improvements, such as microscopes in laboratory instruments or systems, are not included.

In general, the microtechnology scale ranges from 1×10^{-6} to 100×10^{-6} meters; the nanotechnology scale ranges from 1×10^{-9} to $1{,}000 \times 10^{-9}$ meters (i.e., from barely visible with the naked eye to visible only with special microscopes). Typically, microscale and nanoscale devices are manufactured with photolithographic techniques similar to those used for manufacturing silicon computer chips. Manufacturing methods for producing microscale and nanoscale devices out of other materials, such as plastic, usually involve a molding technique.

In most cases, microcomponents and nanocomponents of miniaturized systems must interface to larger systems, perhaps in the millimeter scale or centimeter scale. This requirement must be taken into consideration when comparing the benefits and costs of a device a few millimeters in size to one a few centimeters (hand-held) in size. In some cases, only the micro/nanoscale device can perform the required function. Examples include *in vivo* devices, such as sensors placed in the human body, and catheters, tools that work inside blood vessels.

The laboratory-on-a-chip illustrates how a multifunctional microsystem can eliminate the need for support personnel. If a complex sequence of operations that are currently performed in a laboratory by skilled technicians or scientists can be performed by a package the size of a sugar cube, two benefits will accrue. First, one less person must travel to the field; therefore, one less person must be supplied with food, water, and so forth. Second, miniaturization will save energy in two ways: (1) less weight would have to be transported, and (2) less energy and less reagent would be required for smaller devices.

Microtechnology

In Chapter 3, wristwatch-size sensing and analytical devices were described. To defend against chemical and biological agents, a collection of many highly parallel arrays of miniaturized sensors carried by multiple vehicles and soldiers would be necessary to detect or measure a targeted analyte. Other sensors could be used to monitor the human body, either *in vivo* or on the body surface. The Army could even leverage technologies developed for a laboratory-on-a-chip to combine sensor, laboratory, and antidotal delivery systems on a single, perhaps implantable, biochip, the ultimate defense against chemical and biological agents. Micro/nanosystems may be ideal for performing these sensory and analytical tasks.

The development of easily portable sensor systems capable of reliably detecting and identifying chemical or biological species at trace levels outside a laboratory environment has proven to be a daunting task because of complexity, power consumption, and size and weight issues. The development of operational field units has been hampered by the lack of a commercial demand and the lack of a well-defined requirement by DOD. Even a practical, hand-held system will require a significant engineering effort. DOD and other government agencies have funded the conversion of some laboratory units to field use. Lawrence Livermore National Laboratory and Cepheid have developed a series of field-portable, briefcase-sized or hand-held, battery-operated, nucleic-acid analyzers for detecting bioagents via PCR (polymerase chain reaction) with real-time, fluorescent detection (Woolley et al., 1996).

Multifunctional systems contain one or more key components that provide energy, interface with the environment, transduce signals, process samples, generate information, and communicate with the user. In biotechnological applications of miniature systems, all of these functions must operate in the context of obtaining a biological sample (e.g., fluid), interfacing with the user (e.g., a human), or processing the sample. To improve efficiency and portability, these systems will require miniature components. Component technologies important to miniaturizing biotechnology include fluid-handling devices (e.g., pumps, valves, sampling devices); reaction chambers (e.g., enzyme reactions, ligand binding, synthesis); detectors, (e.g., for target analyte, a physiological condition); and instrumentation (e.g., for information output, feedback, and utilization). Methods and devices will have to be robust, easy to use, and easy to interpret. Work to date has been focused on the development of miniaturized biochemical sensor systems based on MEMS, advances in microfluidics, and microoptomechanical (MOMS) technologies.

Microfluidic Pumping Methods and Actuators

Fluids normally flow in microstructures by laminar flow without turbulence. As a result, layers of fluids containing different components flow together and mix by diffusion only. The mixing resulting from this laminar flow is very rapid. For example, mass and heat transport is 100 times faster when a system is 10-fold smaller, which reduces processing times and enables higher throughput capacities (Bruno et al., 1998).

A variety of mechanical and nonmechanical pumping methods have been used in chips. The most troublesome issues for pumping in microfluidic structures are the avoidance of cavities where fluid cannot flow and bubble formation, which creates back pressure and can inhibit (or even reverse) fluid flow. The fluid pumps in chips work by

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pressure, electronics, or a combination of both. The three primary means of electronic pumping are electrophoresis, electro-osmotics, and electrohydrodynamics.

The most successful pumping in microstructures uses capillary electrophoresis to provide the pumping and valving action that moves solutions, suspensions, or components in solution through microchannels. Both electro-osmotic flow and electrophoresis pumping rely on the conductive nature of the solutions in the channels. The application of voltage along the channel or at ends of the channel creates electrostatic forces on the fluid and components in the fluid, thereby creating electro-osmotic flow and electrophoretic separation, respectively. In the absence of microvalves, electrokinetic pumping has several advantages over pressure-driven flow, especially for analytical separation systems. For example, separation efficiencies are improved as a result of the "plug-like" flow profile of electro-osmotic pumping, back pressure is minimized, and multiple channels on a chip can be readily controlled with a few electrodes (therefore the system has no moving parts).

Both electro-osmotic and electrophoretic pumping methods are well suited to biological assays because most reagents and solutions are aqueous. Many researchers have successfully demonstrated electrophoretic or electro-osmotic fluid management in chips for biological screening (DeWitt and Pfost, 1999; Duffy et al., 1998; Fan et al., 1997; McBride et al., 1998; Mourlas et al., 1998; Studt, 1999; Wallace, 1998).

The third type of electronic pumping, electrohydrodynamic flow, enables the movement of either conductive or nonconductive fluids by the induction of a pressure differential caused by an electric field applied directly in the analyte solution. Both aqueous and organic fluids can be pumped using this method (Boone and Hooper, 1998). This method of pumping, however, has not been optimized and will require additional development before it can be used for applications.

Microreaction Technologies

Not all physical processes can be scaled down. For example, as described above, fluid flow in small-dimension channels is restricted to laminar flow, which precludes mixing by turbulence. Therefore, mass transfer depends on diffusion alone, which can be rate limiting. Analytical sensitivity is also decreased by miniaturization because fewer target molecules are present in a smaller volume. By contrast, heat transfer is improved by miniaturization. Therefore, the development of microreactors for soldier health monitors and internal sensors is very promising.

Many chemical reactions depend on well-controlled environments. For example, in bioreactors for enzyme-substrate reactions used in production of biochemicals, such as vaccines, the reaction parameters (e.g., temperature, pH) should ideally be held constant and should be uniform throughout the vessel. This becomes increasingly difficult as reactions are scaled up in volume. Eventually, it becomes impossible to control the reaction parameters, and as a result poor productivity or, even worse, side reactions occur that can contaminate the reaction mixture. In some cases, volatile or even explosive intermediates can be generated. A growing trend in the development of many parallel microreactions in microfabricated reaction chambers is to scale out rather than scale up parameter-sensitive production processes (Ehrfeld, 2000).

Microreactors may have many other applications:

- uniforms with self-adjusting control mechanisms for such things as temperature, humidity, contamination exposure, energy harnessing, and camouflage
- harvesting of nutrients, energy, medications, and therapies
- medical devices for self-application of drugs
- embedded microsystems to improve the performance of or replace organs and tissues
- synthesis of chemicals and biochemicals
- biosensor detection mechanisms and biofeedback systems

Microreactors coupled with miniaturized sampling, fluidics, detectors, sensors, and computers could enable biosystems capable of solving many key operational problems in the future. In addition, microreaction technologies could be leveraged to improve the performance and capabilities of systems that do not have biological components.

MEMS-Based Microfluidic Systems

A wide variety of microfluidic systems are being developed using MEMS technology. Microflow channels are commonly implemented down to a few microns in size using selective etching on silicon, glass, and plastics. Although many types of integrated microvalves and pumps have been explored to date, progress has been slow, and few if any are commercially available (Petersen, 2000).

Magnetic actuators have been difficult to realize in MEMS because they require materials that are not used in most planar fabrication processes. Electrostatic actuators can develop significant forces only across very small gaps (1μ or less), and the charged surfaces tend to attract particulates, which can clog moving mechanisms. Piezoelectric devices also require nonstandard materials; these devices can produce high force or high throw, but they do not produce both together.

Several thermally actuated microvalves have been reported, but operating power levels are generally high (a watt or more) and performance is modest (Barth et al., 1994; Jerman, 1991). For gas-handling, a device must produce significant force with displacement measured in tens of microns and operating power levels that are consistent with small, portable applications. Thermopneumatic devices, in

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which a solid-liquid or liquid-gas phase transition is used to generate pressure, are among the more promising candidates (Zdeblick et al., 1994). A recent thermopneumatic valve using a liquid-gas transition uses an integrated pressure sensor in the actuation cavity to allow closed-loop control and minimize power requirements. Optimized power levels are in the vicinity of 50mW per valve for a 2,000-torr pressure rise and 1s response time (Rich and Wise, 2000). An integrated microvalve reported for use in a DNA analysis system (laboratory-on-a-chip) is based on the solid-liquid phase transition of wax and generates displacements in the 2nm to 5µm range with high forces (Carlen and Mastrangelo, 1999). The device operates with 50mW to 200mW of power and shows a response time of 30ms.

The development of sample injection and propulsion systems for microfluidic devices, such as DNA processors, is being vigorously pursued (Mastrangelo et al., 1998). To take advantage of the large capillary pressures present in these systems, hydrophobic patches are used to stop the solution flow in the input injector of a DNA processor, and a thermally expanding, electrolytically generated bubble cuts and subsequently propels individual sample droplets (Handique et al., 1997). The device has a sharp neck in the channel to create a surface-induced pressure barrier that stops the flow. Electrolytic bubble generation allows precise metering, and the power dissipation is three orders of magnitude lower than for a thermal drive, making these devices compatible with portable deployment in the field. The capillary pressure barrier that develops when the channel cross-section changes abruptly is in the range of 1kPa to 6kPa, and electrolytically generated oxygen bubbles can be produced with as little as 150µW of power (Man et al., 1998).

A device developed by Burns et al. (1998) is an example of an attempt to reduce laboratory functions onto a chip using micromachining technology. This system has a mixture of micromachined parts and external instrumentation to provide functionality, including microfabricated fluidic channels, heaters, temperature sensors, and fluorescence detectors, to analyze nanoliter-sized DNA samples (see Figure 6-1). The tie-clasp-sized chip includes a nanoliter liquid injector, a sample mixing and positioning system, a temperature-controlled reaction chamber, an electrophoretic

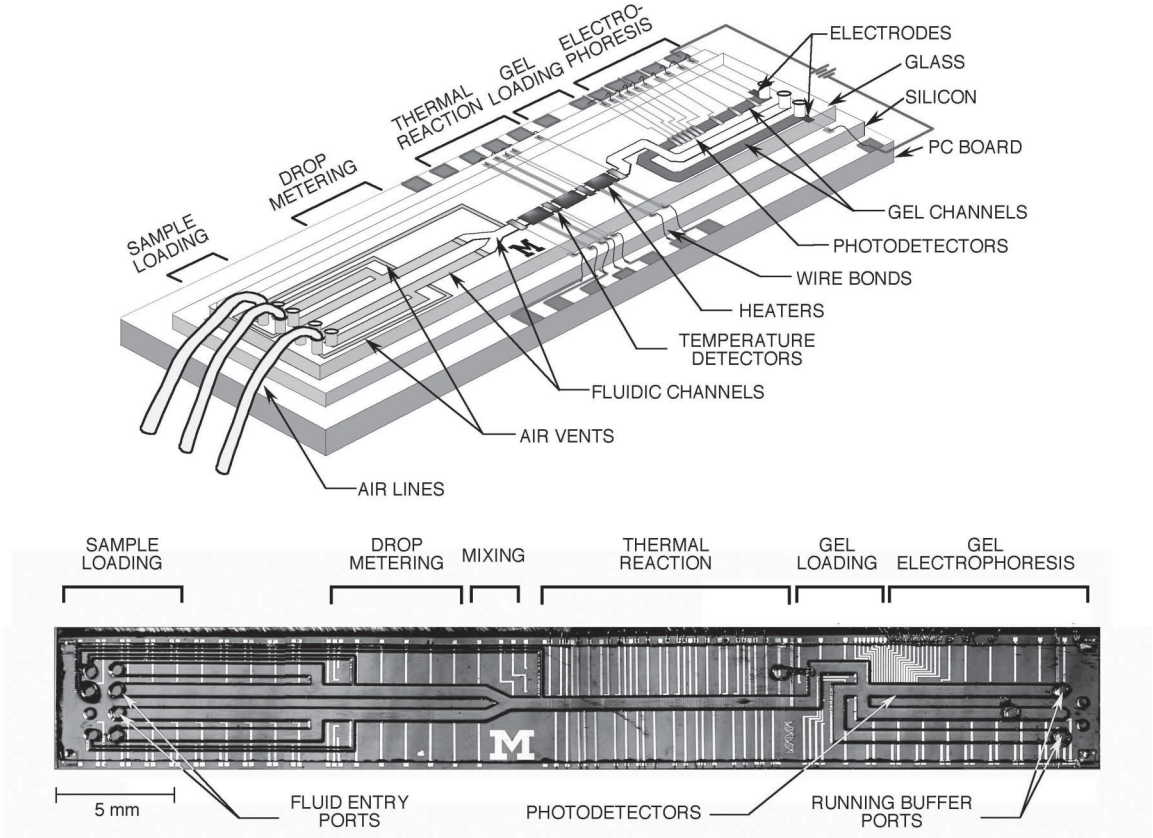

FIGURE 6-1 MEMS device for integrated DNA analysis.

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separation system, and a fluorescence (photodiode) detector. A sample of DNA-containing solution is placed on one fluid entry port while a reagent-containing solution is placed over the other port. Capillary action draws each solution into the device, and the sample is stopped by hydrophobic patches just beyond the vent line in each input channel. Pressure is used to split off precise nanoliter drops, and the DNA and reagent solutions are mixed and moved into a thermal reaction region, where heaters and temperature sensors control the reaction. After the reaction is complete, the sample is moved forward by pressure to the start of a gel electrophoresis channel. The DNA is electrokinetically loaded onto the gel and size fractionated. As the fluorescently labeled DNA migrates through the gel, an external blue-light-emitting diode excites emission, allowing the photodiode to detect DNA concentrations down to 10ng/μl.

Several problems have arisen in miniaturized systems with dimensions measured in microns and fluids in nanoliters: (1) surface tension is so high that fluids can only be moved by large forces; (2) air bubbles can completely block fluid flow; (3) the sample volume is so small that the concentration of the analyte must be very high (i.e., low sensitivity results); and (4) the small dimensions of the device make interfaces to the outside worlds of the sample input and user difficult. Unless these problems can be overcome, systems an order of magnitude bigger (i.e., hand-held systems) will be more useful for analyzing real-world volumes and samples.

The chip mentioned above (Burns et al., 1998) requires an external power supply, a light source, and data analysis electronics. DNA amplification efficiency is limited, and much more development will be required. With significant efforts in packaging, such devices, roughly the size of a pocket calculator, could be available in the next 10 years. To meet its requirements, the Army may want to consider pulling academic work "over the wall" to more advanced prototypes and testing them in the field to accelerate development time.

True integration of miniaturized devices remains a significant challenge with a high potential payoff for the Army. Woolley et al. (1996) showed the successful integration of miniaturized DNA amplification via PCR coupled to microchannel electrophoretic detection of a *Salmonella*-specific assay. The two microcomponents were successfully integrated, and the battery-operated amplification system was the size of a hand-held device. However, a laser-based, fluorescent, confocal microscope of significant size was needed for detection. This laser-based detection system was designed for high resolution, and miniaturization was not considered. With increasingly powerful light-emitting diodes and sensitive miniature photodetectors, DNA fragments can be excited and detected in microchannels. Researchers have detected labeled DNA and peptides in 50μ by 100μ channels; thus, it appears that miniature, low-power detection components are becoming available that are adequate for miniature systems.

Basic research will be necessary to study the fundamental physics and chemistries of sample purification processes and possibly to develop universal sample processing methods. The research would probably have to be focused on different assay types (e.g., DNA test or immunoassay) and determine universal methods for each assay type (see Chapter 3 section on assay formats).

Chip Architectures

Advances in the computer industry have laid the foundation for the integration of fluids with microfabricated chips using silicon, glass, quartz, elastomers, and plastic materials (Hughes et al., 1998). Plastic microfluidic devices offer several advantages over glass or silicon structures, including lower processing temperatures, more options for surface treatment, lower cost, and extensions to multilayer device fabrication. Typically, the fluidic channels in microreactors are 10μ to 300μ in diameter (by comparison, a single strand of human hair is approximately 100μ in diameter).

One significant difference between computer chips and fluidic chips, however, is the three-dimensional nature of fluidic chips. The computer industry typically uses single layers to create electrical networks. The creation of "pipes and valves" to contain and manipulate fluids requires two or more layers bonded or sealed together. More complex fluidic networks combining both horizontal and vertical fluid flows can be achieved in three-layer chips. However, until the advent of microfluidic chips, there was little need for multilayer chips.

Development so far has been driven by the discovery research community, and unit costs have been high enough to preclude evaluation and implementation of new applications. These costs will probably come down when design and production approaches are adopted.

A variety of chip architectures and associated pumping methods have been developed. In general, current chips are two-layer devices operated as linear or branched flow-through systems. The throughput needs of a biological screening program (e.g., 100,000 samples per week) using these chips could be met because of the significantly reduced assay times in the microfluidic environment (i.e., less than 15 s per assay) and using multiple chips in parallel. A design alternative to the flow-through chip developed by Orchid BioSystems is a multilayer device that can parallel process samples simultaneously, analogous to conventional plate-based screening (i.e., 96-well chip).

Overall, however, implementation of chip architectures for biological assays has been limited. Cepheid has developed microfluidic structures that combine a variety of materials, such as micromachined silicon (with an SiO_2 layer) embedded in a plastic cartridge (analogous to a microfluidic

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chip) to extract DNA from biological samples, including samples in the presence of blood plasma (Christel et al., 1998). These microfluidic cartridges have been able to extract infectious bacterial DNA (chlamydia and gonorrhea) from urine, lyse the cells, and reconcentrate, filter, and quantitate the organisms via PCR in less than 30 min (Pourahmadi et al., 2000). This pragmatic, nonmicroscale device is handheld, fits into a portable, breadbox-sized instrument, and is designed to replace a large laboratory system, including several technicians. The relatively large scale is necessary for high sensitivity assays. Reducing this particular system to a truly microsized system may not be possible without sacrificing sensitivity, a significant barrier to miniaturization.

Nanotechnology

The term *nanotechnology* refers to man-made devices and structures with functionally defining elements or components with at least one dimension of 100nm or less. Following federal interagency activities that culminated in a report to the White House, nanotechnology has become a major component of the strategic new research portfolio of the United States (NSTC, 2001). Fundamental research areas in the field of nanotechnology include the science and technology of carbon nanotubes and buckyballs; the self-assembly of chemicals to form tightly controlled supramolecular films and structures; and the direct atom-by-atom assembly of nanostructures by way of manipulation through the tip of atomic-force microscopes. Theoretical models that may be applied in the nanotechnological domain include molecular dynamics and doublet mechanics (Ferrari et al., 1997).

The nanofabrication methods listed above are bottom-up methods in the sense that they feature the construction of structures from atomic-level or single-molecule-level building blocks. Although this approach ensures the greatest latitude for the fabrication of useful constructs, it also encounters major difficulties in the transition from the prototype level to production scale. A different, and probably more scalable, approach involves top-down methods, such as those currently used in microchip processing. For instance, nanopores, which may prove to be instrumental in the development of instrumentation for ultrarapid sequencers, are obtained by a combination of photolithography and sacrificial-layer technologies. Improved sequencing methodologies may also result from a combination of nanotube and atomic-force resolution technologies (Wong et al., 1998).

A very long list of Army-related breakthroughs could result from nanotechnology. Among these are the development of ultrasmall electronic devices, such as single-electron transistors, and nanostructured materials with superior properties. Trends and barriers in nanotechnology developments for bioapplications are described further in Chapter 7 in the section on improved technologies for drug delivery.

Direct Readout of DNA at the Atomic Level

Imagine being able to detect a threat and then obtaining a direct readout of the genetic information (DNA) associated with the threat. Such a technology could not only interrogate microscale particles or molecules to determine friend or foe but could also make an exact identification. This would require the amplification of DNA on a microscale, as well as the sequencing of the DNA once it was available.

Many challenges remain to the direct readout of DNA, including extraction and preparation of the DNA sample and producing overall structures robust enough for use in the field. But direct readout of DNA strands in field-compatible, wristwatch-sized microinstruments is possible in a 20-year time horizon.

The PCR-based process of DNA amplification, separation, and electronic readout has been implemented on a single chip (Burns et al., 1998). In the future, nanotechnology could be used to allow the direct readout/analysis of single strands of DNA at the atomic level at even higher speed (Quate, 2000).

There are several important steps in the development of such devices, and several milestones along this path have already been reached. The first step was the development of scanning surface probes during the 1980s, when it was demonstrated that a sharp tip, brought very close (within < 1nm) to a surface, could allow atomic profiling of that surface through the measurement of tunneling currents or atomic forces between the tip and the sample. The resulting scanning microscopes have revolutionized surface science and are widely used laboratory instruments. Scanning microscopes are also being developed for many applications involving the manipulation of atoms (e.g., in ultrahigh-density data storage systems). These probes are based on micromachined cantilevers with dimensions of a few microns and are fabricated using MEMS technology. A sharp stylus integrated near the tip of the cantilever is used to profile the surface in either contact or noncontact modes. In the case of force measurements, readout is normally done using some form of optical interferometry. An example of a tip and cantilever designed for separately positioned fiber-optic readout is shown in Figure 6-2 (Kong et al., 1993).

A more advanced structure is shown in Figure 6-3. Here, cantilever deflection is measured with respect to an integrated, interdigitated reference plate, allowing very sensitive (0.1Å) determination of cantilever/stylus position. Arrays of these cantilevers can potentially be positioned adjacent to a solid-state imaging array (e.g., a charge-coupled display) and used to read out many points simultaneously. In Korea, cantilever arrays of 780 × 1,000 devices have been realized. The IBM Zurich Laboratory is developing cantilever arrays as a basis for an artificial nose, in which different cantilevers are coated with different materials; the beam bending/stress induced by the adsorption of different molecules is used to identify different gases in the

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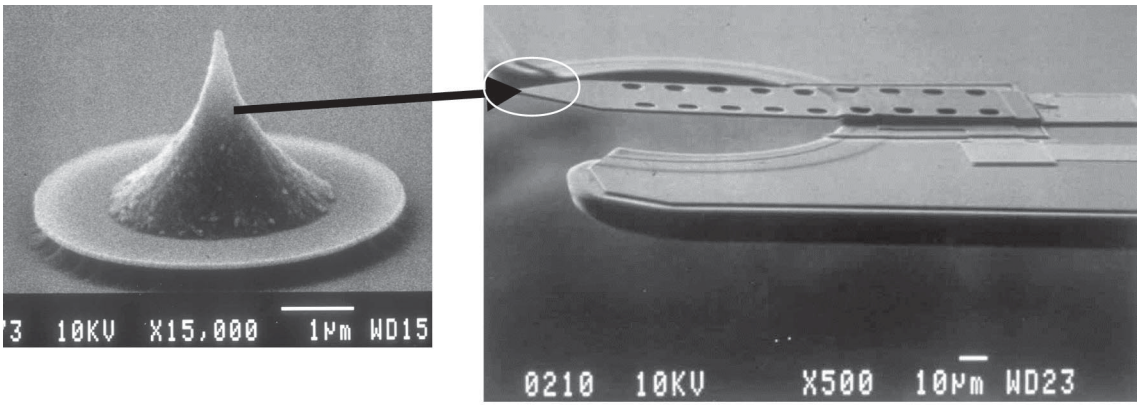

FIGURE 6-2 A micromachined cantilever and tip-mounted stylus designed for readout using a separately positioned fiber-optic reference.

Source: Reprinted with permission from Kong et al., 1993. Copyright 1993, Journal of Vacuum Science and Technology.

ambient air (Lang et al., 1999). Cantilever bending by oligonucleotide hybridization has also been demonstrated.

A second milestone in the development of atomic-scale DNA chips is the ability to detect extremely small amounts of charge. Snow and Campbell (1995) have shown that very thin titanium films can be selectively oxidized using a scanning probe to create tunneling junctions, and K. Matsumoto et al. (1996) of the Electrotechnical Laboratory, Tsukuba, Japan, has used this technique to form single-electron transistors (the most sensitive electrometers), as illustrated in Figure 6-4. When fabricated with dimensions of 10nm, these devices can operate at room temperature. It is,

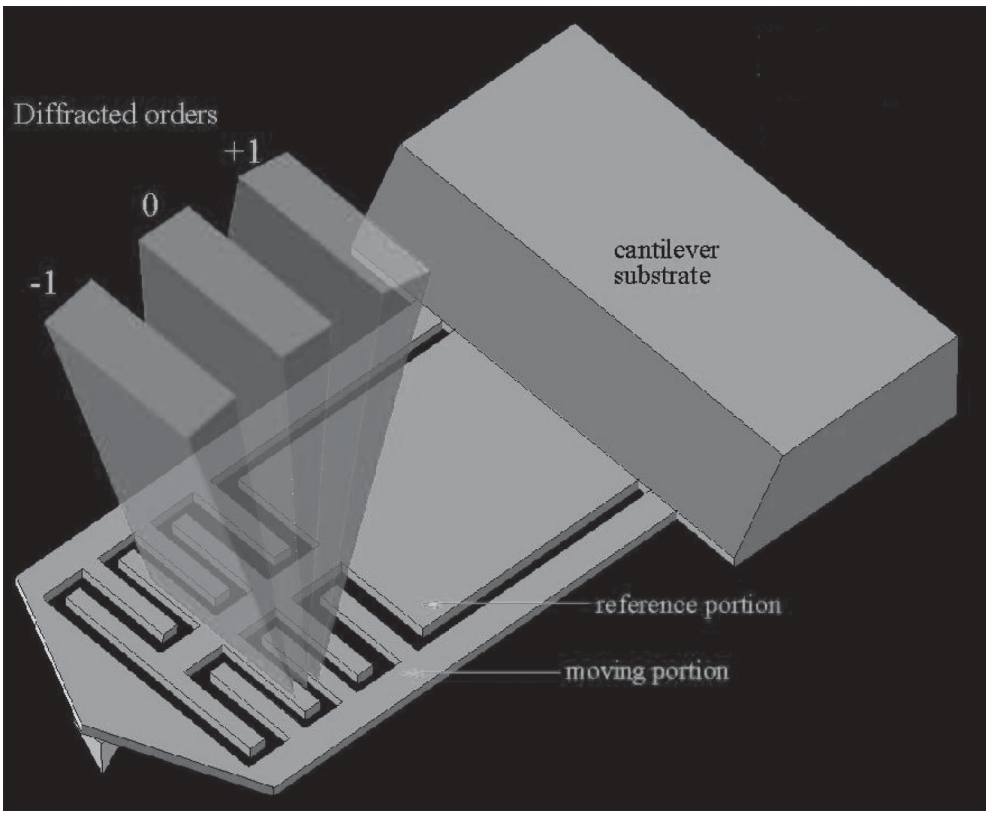

FIGURE 6-3 An interdigitated cantilever and fixed-reference plate forming a scanning surface probe. The cantilever position can be measured with great accuracy using diffraction.

Source: Quate, 2000.

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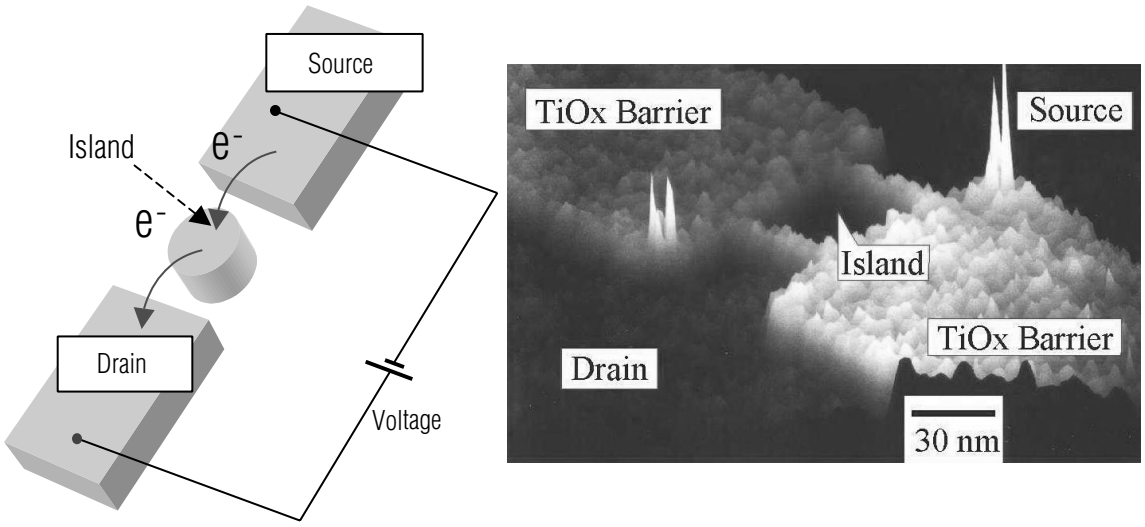

FIGURE 6-4 Schematic drawing and structure of a single-electron transistor for measuring electronic charge.

Source: Quate, 2000.

therefore, possible to think in terms of integrating single-electron electrometers with scanning cantilevers to measure the charge on single DNA strands as they are lowered past the tip (e.g., pulling them through 1.5–2nm holes, such as has recently been demonstrated in silicon nitride membranes). The use of single-wall carbon nanotubes has been proposed for the integration of single-electron transistors directly into the stylus tip of the probe. (Nanotubes are perfect quantum conductors in which adjacent molecular interactions effect conductivity.)

Key Recommendation

The Army should support basic research in microfluidics and nanotechnology that will facilitate the miniaturization of sensing capabilities for both internal (*in vivo*) and external (*in vitro*) applications. In addition to supporting the development of new Army systems and reducing logistics, micro/nanotechnologies could potentially benefit other Army systems that will perform a wide range of missions.

FUNCTIONAL FOODS

Functional foods are foods or ingredients with components that influence metabolism or physiology beyond ordinary nutrition. Functional foods can contain nutraceuticals, which are both nutrients (vitamins) and physiologically active compounds, and/or phytochemicals, which act as health protectants. Phytochemicals are plant-derived compounds purported to afford specific health benefits (Watkins, 2001).

A critical barrier to the development of functional foods may be the public perception that genetically modified organisms are undesirable. The development of crops with higher levels of natural pesticides reduces the need for chemical pesticides. Although public resistance in the United States has been relatively minimal, resistance in Europe has been widespread due in part to the incidence of prion diseases, such as mad-cow disease, which have been traced to alterations in the beef processing chain in Great Britain. Other barriers to the development of functional foods include maintaining palatability and cooking characteristics while introducing the new functions.

Crops with enhanced levels of nutritional components, built-in vaccines, or edible factors that impart resistance to spoilage are in the offing. The benefits to the Army of functional foods are numerous, and some foods could be developed to meet specific Army needs. Foods that could reduce the incidence of dysentery, for example, could significantly increase the readiness of Army personnel. Rations could be formulated to contain edible enzymes that are natural, tasteless, and nontoxic, to aid digestion. More efficiently digested foods would mean that more calories could be transported to troops for the same amount of weight.

Foods with built-in, naturally occurring, antimicrobial factors could inhibit the pathogenic microorganisms soldiers might be exposed to in the field. Anti-infective properties might be based on edible proteins or peptides. Foods could also be optimized for safety and storage (Linton, 2001). If the need for refrigeration of some types of fresh fruits and vegetables could be reduced or eliminated, it would reduce the need to transport and power refrigeration equipment.

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More efficient and complete digestion might also reduce the amount of food a soldier must carry because less food would be providing the same amount of energy. NASA-sponsored research for human space flight suggests that significant reductions may be possible (Dunbar, 2000).

Providing a means of identifying people as friend or foe is critical to battlefield and peacekeeping functions; in combat, combat identification should be possible at great distances, perhaps with remote sensing. The presence of particular biological organisms or attributes could be used to help identify, trace, or track individuals. Biological tagging of soldiers could also be accomplished by feeding soldiers foods containing biomarkers, thus distinguishing friendly soldiers from the enemy. Being able to distinguish among friendly forces and units could also significantly improve command and control.

Agriculture will be an essential component of the emerging biotechnology industry, both as an end user of technological advances and as a supplier of the carbohydrates and other biological materials. Agricultural biotechnology can also play a direct role in reducing the logistics burden. Rapidly growing plants could provide in-theater sources of food, fuel, or energy. Perhaps infrared or radar-absorbing properties common to vegetation in the field could be used to grow new materials that would make troops undetectable by enemy sensors. In effect, these would provide a native camouflage.

Genetically Engineered Foods

Genetically modified seeds have had a great impact on agriculture. Corn and cotton modified by *Bacillus thuringiensis* (B.t. corn and B.t. cotton) for example, have greatly reduced the amount of pesticides that are required during growth cycles. Genetically modified soybean seeds resistant to herbicides have also been introduced. Monsanto and other companies have made significant investments in the development of these foods. Although most of these agricultural developments are not appropriate for Army investment, the impact of these developments should be carefully monitored, particularly for the availability of biomaterials, like food and fuel, that the Army might be able to use.

The genetics of plants and animals have been manipulated for centuries for the purpose of amplifying positive characteristics ranging from robustness of the organisms when faced with a range of environmental conditions to enhancement of the ratio of edible to nonedible portions. The commercial potential of genetic manipulation using recombinant methods has only recently begun to be realized; at the same time significant public resistance has arisen to genetically modified organisms. Nevertheless, research continues, and foods with amplified functions are being developed.

Some new foods are being developed that are only achievable using genetic engineering techniques. Research by and for the U.S. space program has provided insight into many potential applications (Kohlmann et al., 1996; Mitchell et al., 1995; Velayudhan et al., 1995). In the future, plant converters could take energy from sunlight and carbon dioxide from air and convert them into required materials in days rather than weeks. A pocketful of seeds could become the slow-motion equivalent of a Star Trek type "replicator."

Edible Vaccines

Edible vaccines are a very good example of functional foods that could greatly simplify the logistics of vaccinating soldiers. The purpose of a vaccine is to prepare the immune system to destroy specific disease-causing microorganisms before they multiply sufficiently to cause symptoms. Priming the immune system against possible invaders is typically achieved by presenting whole viruses or bacteria that have been killed or weakened to the immune system. This causes an acute response followed by the establishment of memory cells that remain on alert to mobilize the immune system if a real pathogen enters the body. Some vaccines provide lifelong protection. Others must be readministered periodically.

Some vaccines pose a slight risk of propagating and causing the diseases they are meant to forestall. Subunit vaccines avoid this risk because they consist of antigenic proteins separated from the parent cell's genes and the parent cell itself. A subunit vaccine does not form the pathogenic organism from which the protein is derived. Charles Arntzen is credited with the concept of genetically engineering foods to be like subunit vaccines when the food is eaten; that is, the protein that induces immunity is separated from the pathogenic organism from which it is derived (Langridge, 2000). See Appendix D for detailed discussion of subunit vaccines.

Edible vaccines in foods seem especially appropriate for combating diarrhea. The causes of this affliction include the Norwalk virus, rotavirus, *Vibrio cholerae* (the cause of cholera), and enterotoxigenic *Escherichia coli* (a toxin-producing source of "traveler's diarrhea"). Mucosal membranes that line the digestive tract, the first line of defense against these pathogens, generate proteins (referred to as secretory antibodies) that are secreted into the cavities lined by the membranes and that play a role in neutralizing pathogens. A systemic response to a pathogen adds to this protection by circulating cells that destroy pathogens that may have passed through the membrane. Because edible vaccines would contact the lining of the digestive tract, in theory, they could activate both mucosal and systemic immunity.

Challenges being addressed by ongoing research include engineering plants so that their edible part carries genes that express specific proteins when the plant is grown; packaging proteins in food so that they are not destroyed by the digestive system; and identifying and confirming that the proteins generate an effective response. Edible vaccines would be attractive, easy to administer, and, presumably, would elimi-

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nate the need for the refrigeration and delivery through injection of current vaccines. The Army should consider a number of logistical questions, such as if rations intended to prevent different diseases would have to be kept separate from other foods and how to keep track of which members of a unit had been vaccinated. Commercial challenges will also have to be overcome. As of 2000, no one had produced an edible vaccine for sale (Langridge, 2000).

Key Recommendations

Agricultural biotechnologies can be beneficial for the Army beyond improving nutrition. Engineered foods, edible vaccines, and biological tagging are all near-term technologies that could increase soldier effectiveness, improve command and control, and reduce logistics support requirements. The Army should build on developments and research at its Natick Research, Development and Engineering Center and should take the lead in developing new and innovative functional foods that provide high nutrition, are lighter, can be stored longer, and incorporate therapeutic or prophylactic properties.

The Army should monitor developments in the field of plant biotechnology, because significant private and government sector investments will result in enhanced (transgenic) crops, functional foods, and plant-derived biomaterials. The Army may be able to leverage these investments by industry by taking advantage of new developments once they have been field-tested and received public acceptance.

BIOLOGICAL PHOTOVOLTAICS

Almost all of the energy the Army uses today comes from fossil fuels and batteries that must be transported to and distributed on the battlefield through the logistics system. As military forces become smaller and lighter, and as fuel sources for society as a whole change, a high proportion of the Army's future energy needs may be satisfied by renewable resources. Biological photovoltaics may also help to meet energy requirements for individual soldier electronics in the field.

Plants and algae convert sunlight into energy with a quantum efficiency of about 98 percent. Mimicking plant energy-conversion processes could provide a basis for solar-derived power for use on the battlefield at operational efficiencies competitive with semiconductor solar cells. Although not enough research has been done on biological photovoltaics to provide useful data on operational efficiencies, when the quantum efficiency approaches unity, the best solar cells can theoretically approach a maximum of 50-percent operational efficiency (with the system matched at maximum power point). Because system-coupling losses limit the operational efficiencies, semiconductor photovoltaic converter systems typically operate in the range of 10 to 15 percent.

Biological photovoltaics may provide optimal coupling to the radiation field. Natural selection has already optimized plant and bacterial light-harvesting systems to couple efficiently with the solar spectrum so that many of these systems operate with quantum efficiencies approaching unity. Thus, it is not unrealistic to hope that these systems could be harnessed to produce devices with operational efficiencies approaching 40 to 50 percent. In addition, if biological photovoltaic devices can be made to mimic the responsivity of plants in both the infrared and visible regimes, the probability of enemy detection of large solar converters would be minimized.

Photosynthesis

Photosynthesis is the process by which green plants use the energy of sunlight to manufacture carbohydrates from carbon dioxide and water in the presence of chlorophyll. The initial electron-transfer (charge-separation) reaction in the photosynthetic reaction center sets into motion a series of reduction-oxidation reactions, passing the electron along a chain of cofactors and filling up the "electron hole" on the chlorophyll (the so-called bucket brigade). All photosynthetic organisms that produce oxygen have two types of reaction centers, photosystem II and photosystem I (PSII and PSI, for short), both of which are pigment-protein complexes located in specialized membranes called thylakoids. In eukaryotes (plants and algae), these thylakoids are located in chloroplasts (organelles in plant cells) and are often found in membrane stacks (grana). Prokaryotes (bacteria) do not have chloroplasts or other organelles, and photosynthetic pigment-protein complexes are either in the membrane around the cytoplasm, in invaginations thereof (for example, in purple bacteria), or are in thylakoid membranes that form much more complex structures within the cell (most cyanobacteria). Photosynthesis, in general, is the reverse of respiration, a process of breaking down carbohydrates to release energy.

Biomolecular Diodes

Photosynthesis in plants is initiated by the absorption of light by the two specialized reaction centers, PSI and PSII. The absorption of a photon triggers rapid charge separation and the conversion of light energy into an electric voltage across the reaction centers. These complex protein systems are about 6nm in size and can be isolated and incorporated into devices to generate biomolecular diodes. This research, being carried out Oak Ridge National Laboratory, is summarized in Figure 6-5 (Lee et al., 1997).

The value of biomolecular devices is in their potential, rather than their current realizations. Through genetic

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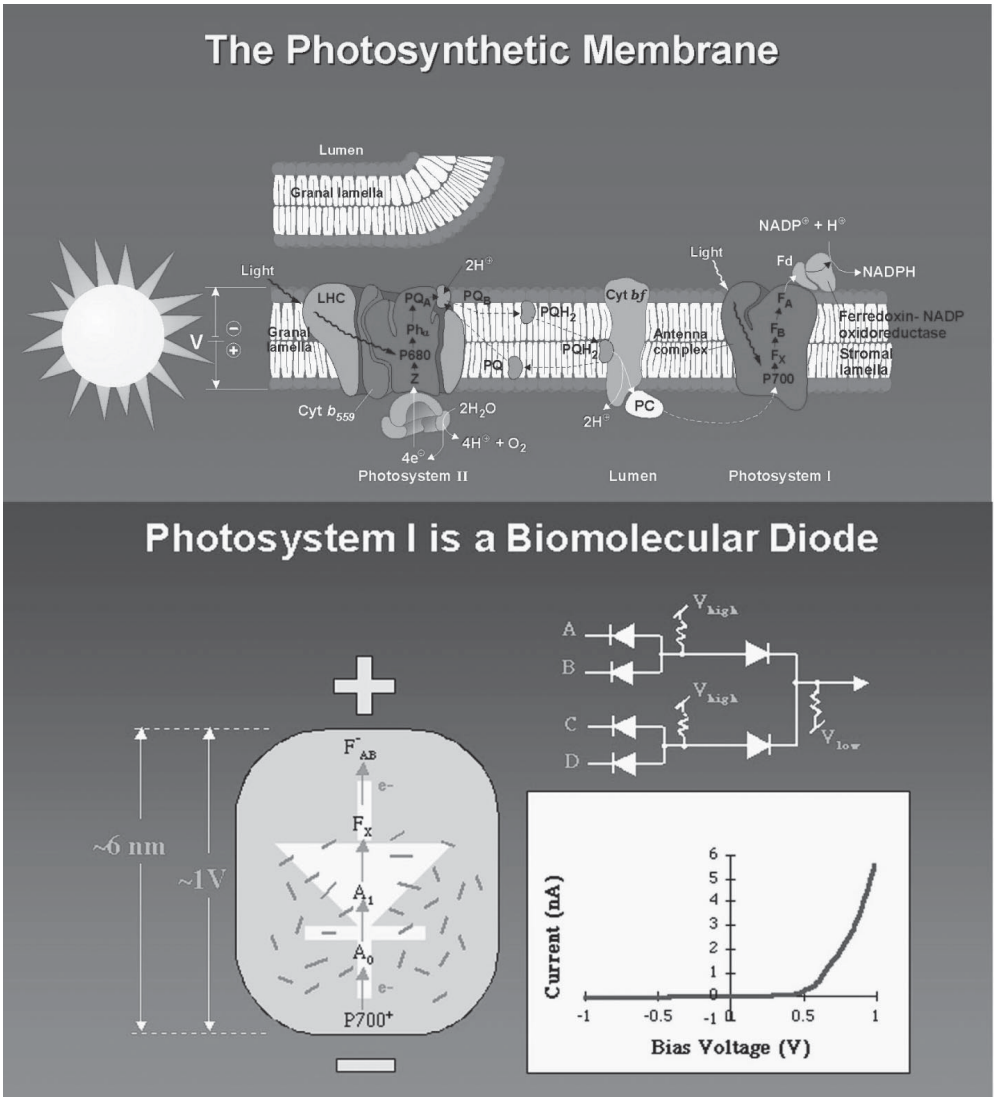

FIGURE 6-5 The photosynthetic apparatus of plants converts light into energy with high efficiency. The isolated Photosystem I can be used as a biomolecular diode, as shown in the lower inset.

Source: Reprinted with permission from Lee et al., 1997. Copyright 1997, American Physical Society.

engineering, the voltage-response characteristics could be adjusted. Considering that PSI and PSII operate at efficiencies of 70 percent or more and are tuned to intercept solar radiation with optimal absorptivity, further engineering might produce the ultimate photovoltaic converter. These hybrid systems, which currently have operating lifetimes measured in days instead of years, will have to be stabilized.

Isolating the photosystems and using their ability to convert light into an electron gradient as a photovoltaic device is not a simple task. These pigment systems were not designed just to convert light into electron translocation, but rather to use light for electron transfer simultaneously with the conversion of water to oxygen plus a proton (PSII) or to convert a light-induced electron gradient to convert the electron acceptor NADP (nicotinamide adenine dinucleotide phosphate) (PSI). Any photovoltaic system must either provide a superstructure to make these reactions possible or mimic their behavior to maintain functionality.

The U.S. government's investment of more than $1.5 billion in the past 25 years, combined with more than $3 billion invested by the private sector, has resulted in only a small improvement in the efficiency of semiconductor-based photovoltaics. Multijunction cells based on GaInP/GaAs have achieved efficiencies as high as 30 percent, but these cells are very costly. Cost-effective amorphous silicon is still below 15 percent in efficiency. Relatively little funding has been directed toward photovoltaics based on biomolecular systems.

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In light of the potential payoff, and despite the problems discussed above, it would seem prudent for the Army to investigate the potential of bioelectronics for photovoltaic conversion. If photovoltaic conversion were combined with camouflage, it could provide as much as 20mA at 12V from the surface of a protein-based photovoltaic coating on a Kevlar helmet. Full sunlight is not required, and gray-day photovoltaic converters could be combined with energy-storage mechanisms in regions where sufficient sunlight is not available. The power could then be used directly or used to recharge batteries in the field, thereby increasing the range of an operation and decreasing the soldier's load.

Advances in self-assembly techniques suggest that biomolecular photovoltaic paints could be developed to provide inexpensive, rapidly deployable, photovoltaic power for Army systems in the field using photosynthetic principles. It is unlikely that such technologies will be investigated without sponsorship from the Army. The high potential of biological photovoltaics provides ample rationale for Army research on making the photosynthetic apparatus of plants or bacteria compatible with current electronic storage systems.

Key Recommendation

Biological photovoltaics is a promising technology that could satisfy Army power requirements in the field, but usable products are at least 15 years away. Photovoltaic converters with superior efficiency are based on bioelectronics devices, such as hybrid diodes and transistors. The voltage-response characteristics in these devices may be adjustable by genetic engineering. The long-term potentials, therefore, will depend on intervening advances in genetic engineering and photovoltaics research. The Army should monitor the progress of research in genetic engineering and photovoltaics for new developments that could meet the energy requirements of soldiers in the field.

RENEWABLE RESOURCES

In 1998, a vision was articulated through a study led by the Office of Industrial Technology of the U.S. Department of Energy "to provide continued economic growth, healthy standards of living, and strong national security through plant/crop-based renewable resources that are a viable alternative to the current dependence on nonrenewable, diminishing fossil resources." This vision responds to the rapid increase in total resource consumption and sets a goal of deriving at least 10 percent of basic chemical building blocks from plant-derived renewable resources by 2020 (DOE, 1999).

The Army may be able to benefit from the research efforts expended in pursuit of this vision. For example, biologically derived fuels may provide practical alternatives to gasoline and diesel. Also, plant-derived indigenous materials might be used to satisfy requirements for food, fuel, or other high-volume consumables during extended operations.

Renewable Fuels

The primary motivation for investigating renewable resources as a replacement for oil is that the United States now imports over 50 percent of its oil. Continuing research is also attributable to environmental regulations for clean air and transportation fuels, renewed concern about the security of petroleum supplies, and continuing government subsidies.

The tailoring of plants to give specific products is now possible due to the advances in transgenic plants. Biodiesel is a fuel derived from soybean (or other plant) oil that has been demonstrated to replace effectively petroleum-based diesel fuel. The Army uses both gasoline and diesel on the battlefield. Various blends of fuel, including those containing as much as 85 percent ethanol, have also been shown to be effective replacements for gasoline.

The benefits of alternative fuels, which are currently very expensive, are simpler methods of processing and availability wherever there is vegetation. Small grains, grasses, even agricultural residues, can be converted to ethanol via fermentation, and oil seeds can be extracted to obtain lubricant oil. The effective yield of fuel alcohol would vary depending on crop yield and the type of crop, but the committee estimates that the yield would range from 100 to 400gal per acre.

Biofuels could provide the Army a measure of independence from extended fuel supply lines. However, to take advantage of this capability, the Army must keep abreast of developments in conversion technologies, prepare conversion units, and test them to ensure that military engines can use both traditional and alternative fuels. The Army could also develop expertise in conversion bioprocesses so that the production of alternative fuels could be quickly initiated, as needed.

As the potential for biologically based production grows, so will the requirement for engineering. Bioprocess engineering (the discipline that deals with the development, design, and operation of biologically based processes) will play a major role in the development of bioprocesses for transforming renewable resources into useful chemicals and products.

Progress is being made in pretreatment and enzyme hydrolysis, as well as in obtaining genetically engineered microorganisms that can convert both pentoses and hexoses to ethanol; however, conversion processes used in these technologies will require major improvements to become economically viable (Gulati et al., 1996).

Specialty Products

The emergence of a cellulose-based biofuel industry in the United States would open the way for the production of

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many other types of oxygenated chemicals with potential Army applications. Concerns about pollution and global warming have driven the development of fuels and chemicals that are less likely to cause buildups of carbon dioxide or ozone-depleting substances in the atmosphere. Renewable resources that contain cellulose or starch are promising because the products derived from them are eventually broken down into carbon dioxide (e.g., combusting a fuel), which is recycled back into the plant via the Calvin cycle. The conversion requires sunlight as its source of energy.

Waste cellulose materials, such as grasses, agricultural residues, wood chips, surplus grains, spoiled surplus food, and food wrappers, could be used as sources of sugars. These sugars could be fermented to chemicals that contain oxygen and could be used as building blocks for plastics and fabrics. In theory, these materials could be produced in the field (if the theater of operation were in a temperate zone) and used as fuels.

The mass production of genetically engineered plants by modern agriculture in the United States could accelerate the development of specialty polymers or materials for soldiers' uniforms. Materials from genetically engineered plants could be readily processed into fabrics, either directly or indirectly. Natural polymers could combine the best characteristics of cotton and artificial fibers to perform specialized functions to protect soldiers from the environment.

Some products from renewable manufacturing sources will certainly be available before 2025, driven largely by consumer environmental concerns, international conventions on global warming, and the attractiveness of "natural" manufacturing processes that decrease the use of chemicals perceived to be undesirable because of their toxicity or other handling characteristics.

Life-Support Applications

Perhaps the ultimate application of biotechnology will be to control ecological life-support systems in an alien environment, such as space, or in the confined environments that may be encountered by soldier operators of future Army combat systems. Biological life support will also be necessary for long-term space travel and, particularly, for the colonization of Mars. Food will have to be generated and wastes recycled to support life (Westgate et al., 1992). Controlled, bioregenerative, life-support systems would mimic Earth's ecosystem, recycling carbon, hydrogen, oxygen, and nitrogen through a complex, interconnecting series of biological and biochemical transformations (Averner et al., 1984).

The technologies that result from research and development in these areas can be applied directly to the support of military operations for prolonged periods of time in remote locations. They will also add to our understanding of Earth's ecosystem and provide solutions to environmental issues, such as pollution control and remediation. The Army is responsible for controlled destruction of chemical munitions and could benefit greatly from research in this area, especially bioremediation, the biological conversion of toxic wastes to nontoxic materials.

Key Recommendations

As biologically derived alternatives (surrogates) for gasoline and diesel fuels become available, they could provide increased flexibility for future Army operations. The Army should monitor the development of bioprocesses for alternative liquid fuels. Simultaneously, the Army should prepare to test and adapt military engines so that they can operate with either traditional or alternative fuels.

Biological methods of recycling air, food, and water could improve Army systems that require soldiers to work in confined spaces for extended periods of time and could decrease the logistical support requirements of soldiers in the field. Many such methods are already under development in response to commercial and NASA requirements. The Army should identify conditions specific to its needs for operating future combat systems and monitor developments in biological life cycle support applications.

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7

Soldier Health and Performance

Many developments in biotechnology will initially be intended for medical applications. Over the next 25 years, the pharmaceutical and biotechnology industries will make enormous investments in technologies to translate information uncovered by genomics into knowledge of disease pathways and targets; that knowledge will then be used to develop novel therapeutic compounds and vaccines. This chapter examines developments in therapeutics and genomics that are expected to be important to the Army as a whole, as well as to individual soldiers.

Based on current trends, vaccines and therapies will soon be tailored to suit individual soldiers. New technologies may lead to dramatic increases in the development of new drugs and vaccines and in equally dramatic reductions in the time and cost of developing them. For the Army, these reductions in time and cost will provide opportunities to develop therapeutics and vaccines against diseases that are not of commercial importance but are endemic to areas where forces may be deployed.

If current trends continue, predicting and designing drugs to augment individual performance will be possible. Unlike previous wars in which dozens of soldiers were needed for each kilometer of front, in future wars there may be only one or two soldiers per kilometer of front or 10 to 15 soldiers per square kilometer in shifting combat zones with no fronts (Rhem, 2000). In these wars, fewer warfighters will have increased responsibility for each battle. With advances in our understanding of biological processes, the effects of combat stress might be mitigated and the survivability of warfighters increased.

GENOMICS

The term *genomics* was first used to refer to information of interest to industry about DNA, including sequence information. The term *functional genomics* has come to refer to information about what genes do, especially information about RNA and protein products of genes, often called *proteomics*. In this report, all of these are included in *genomics*, and this whole area of research is called *genomic biology*.

In principle, genomics provides a means of identifying, in any cell, tissue, or organism, all of the important genes and regulatory regions in the DNA, all of the mRNAs, and all of the proteins in different states of cell and organ function. Genomics has transformed the science of biology by enabling the discovery of new links between protein structure and function (see Box 7-1).

Almost as a by-product, genomics also provides means of identifying differences among different individuals at the level of their DNA, RNA, proteins, and other expressed molecules and of determining the significance of these differences. This information will be valuable for correlating differences with particular outcomes, gaining insight into the biological mechanisms caused by or affected by these changes, and suggesting grounds for subdividing, or stratifying, populations such as soldiers. Therapies and enhancers could even be tailored for individual soldiers to accomplish specific ends.

Genomics Information-Gathering Techniques

For practical purposes, DNA information does not change during the life of the organism. By definition, this information can be strongly or weakly predictive of behavioral characteristics (see section below on prediction and enhancement of soldier performance). By contrast, mRNA and proteins change in response to events inside and outside the organism and can be used to predict events that occur over long (year to year) or short (hour to hour) time frames. Genomics information can be gathered through techniques involving DNA, RNA, and proteins.

DNA Techniques

One means of gathering information about DNA is to sequence it, which means determining the nucleotide sequence

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BOX 7-1
Determining Function Through Protein Structure

The central dogma of genomics is that sequence determines structure determines function. Sequence applies to DNA sequences, and structure applies to the structure of the proteins encoded by the DNA. As more and more gene sequences are identified, more information is becoming available for deriving the functions of gene products.

One way to determine the function of genes ought to be by protein structure, because the overall fold of a protein should correlate with, at the very least, its biochemical function. Thus, proteins possessing a Walker ATP-binding motif should bind and hydrolyze ATP or some other nucleoside triphosphate; proteins with an EF-hand should bind calcium ions and use this binding as a conformational switch, and so forth.

This reasoning is the basis for the new Structural Genomics Initiative, a concerted effort by dozens of structural biology laboratories around the world to determine at least one three-dimensional structure—by either nuclear magnetic resonance (NMR) spectroscopy or, more commonly, by x-ray diffraction methods—for every type of polypeptide chain fold. Reasoning that the availability of a "fold library" would enable the structures of any homologous protein to be built by simple modeling using the representative structure from the library as a template, proponents of this initiative hope to determine 2,000 to 5,000 structures a year until the catalog of folds is complete.

Some computational biologists are attempting to solve protein structures by predicting them directly from the amino acid sequence. These efforts are of two main types: (1) recognizing the fold from homology with proteins of known structure and (2) folding of linear polypeptide sequences *in silico* to produce a three-dimensional model of the protein. The former technique, which is already well established, constitutes a large part of what is commonly termed bioinformatics. The technique of folding a protein sequence directly into its structure, however, has a checkered history. Most "successful" attempts have produced atomic models that only grossly resemble actual structures, with no possibility of the model being useful for drug design or functional studies. These methods involve using empirical energy potential functions to manipulate a set of atomic coordinates in a computer to attain a state of lower empirical potential energy. The underlying assumption is that the global minimum of this function will be the correctly folded structure.

Simulations of protein folding require enormous amounts of computer time even though the time scales are generally much faster than the time actually required for a protein chain to fold up *in vivo*. Recent efforts have focused on the development of massively parallel supercomputers, such as IBM's Deep Thought computer, to simulate folding on more nearly physiological time scales. However, it is unlikely that this will provide a simple, near-term solution to the problem of predicting direct structures.

Improving algorithms for recognizing protein-protein association sites from sequence information seems to be worthwhile, as is the development of improved empirical potential-energy parameters for proteins. In the short term, however, experimental determination of protein structure will almost certainly greatly outpace *de novo* structure prediction, at least for proteins with no obvious homology to proteins with known structures.

Important work being done in this area and enabling technologies include the development of functional databases; computational tools that analyze three-dimensional structures for small-molecule and protein-binding sites; and computational approaches to the recognition of catalytic motifs in protein folds. Determining the structures of several thousand proteins per year is a formidable challenge, but the methods to meet the challenge are nearly in hand. Lack of access to synchrotron radiation, the high cost of multiuser facilities, and the need for new materials are the main obstacles. Increasing access to synchrotron radiation will require equipping new beam lines for high-throughput protein crystallography and providing personnel to operate crystallographic beam lines.

A central facility for the large-scale cloning, expression, and purification of proteins from human cells and pathogens could serve as a resource for the entire structural biology community. Computational tools that can solve the phase problem in protein crystallography and automated electron-density map interpretation will also be necessary. Because not all proteins can be crystallized, high-throughput nuclear magnetic resonance (NMR) initiatives may be helpful for increasing the rate of production of new structures; especially interesting would be using x-ray and NMR techniques in complementary ways.

Source: Petsko, 2000.

of contiguous stretches of DNA up to the entire genome of an organism. Now that the DNA sequences of many organisms, including humans, are known, a treasure trove of biological knowledge, including insights into evolutionary history and biology has been revealed. The implications have barely begun to be realized.

The cost of sequencing has dropped tenfold in the past five years to about $0.20 per base pair in 2000, and the development of enabling technologies is still accelerating. At that rate, the cost will have dropped another five orders of magnitude by 2025. By that estimate, it will cost $1 to sequence 500,000 base pairs and $6,000 to sequence an entire human genome. In other words, by 2025, comparisons of complete DNA sequences between individuals and reference sequences may have become routine.

At the moment, although it is not economically feasible to determine complete sequences of human individuals, it is possible to identify differences among DNA sequences by a

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number of methods. These differences are referred to as polymorphisms, or nucleotide polymorphisms. If two DNA sequences differ at a single position, the difference is referred to as a *single nucleotide polymorphism*, or SNP (pronounced snip) (see Box 7-2).

Information about polymorphisms is generated by a growing list of technologies ranging from (1) PCR with selected sets of oligonucleotide primers to (2) differential hybridization to (3) photolithographically synthesized arrays of oligonucleotides on chips to (4) ligation chain reaction to (5) the use of cycles of polymerization oligonucleotide primer resulting in DNAs of different lengths that can be analyzed using time-of-flight mass spectrometry.

SNPs and other nucleotide polymorphisms are especially valuable markers for identifying function or behavior expressed by an individual because they can be easily, and accurately, detected. SNPs and other genetic markers have advantages over phenotypic markers because it is easier to identify many different markers in DNA from two different individuals than to determine an equivalent number of genetically determined phenotypic traits, such as eye or hair color. The Army commonly uses DNA as an aid in the identification of soldier remains; in the future it may become possible to use distinguishing characteristics of DNA as a biometric for secure access (e.g., to identify conclusively soldiers authorized to use classified information).

Although these technologies are too new to establish a trend line with the same confidence as for sequencing, it seems inevitable that the cost of determining a set of SNPs in an individual (i.e., *genotyping* the individual) will also drop

BOX 7-2
Single Nucleotide Polymorphisms

The easiest way to introduce the concept of single nucleotide polymorphisms (SNPs) is to think of the different meanings of the word *gene*. To molecular biologists, for the last 50 years, genes have been the stretches of genomic DNA, encoded proteins, that are components of cells and organisms. This definition of a gene (i.e., something that encodes one of the component parts) has largely superseded an older definition based on work by Mendel and made rigorous by Morgan, Sturdevant, Müller, and others in the early twentieth century. In the older definition, a gene was a unit of heredity (i.e., something that encodes a detectable property of an organism). That property is called a *trait* or, nowadays, a *phenotype*. The older definition of a gene only makes sense operationally because one can determine if a trait is present (*score* a phenotype) if the phenotype comes in at least two distinguishable configurations— tall or short, blue-eyed or brown-eyed, sickle cell or non-sickle cell, dimple or no dimple. Classical genetics depends on these scorable differences.

Different forms of a gene that give distinct phenotypes are referred to as *alleles*, and genes are said to exist in different *allelic states*. For example, say that a man has inherited a trait, pattern baldness, from his father or mother. The relevant gene might encode a serum testosterone receptor found in hair follicles, and the man may have inherited the pattern baldness *allelic form* of the gene, which might, for example, encode a form of the testosterone receptor that causes the hair follicles to die after exposure to serum testosterone for 20 years.

The fact that one has pattern baldness means that one has inherited DNA from one's mother or father that carries the pattern baldness allelic form of the gene. Because the pattern baldness allele was located on a chromosome next to many other genes, if one has the pattern baldness allele from one's father, one is also likely to have the same allelic forms of genes near that allele in one's father. Those traits thus *cosegregate* and are said to be *linked*.

The cosegregation of traits (linkage) also occurs in the population as a whole because the human species is still young. Most estimates suggest that humans are no more than 80,000 years old, or only 3,200 generations removed from a founder population in Africa. The small number of generations means that an individual who has a specific allele also has a high probability of having other specific alleles at nearby genes. So the presence or absence of a particular marker, such as a SNP, is a strong predictor of the allelic state of nearby genes. This fact allows us to look at the SNP and guess about the state of the neighboring genes, rather than having to isolate the neighboring genes and sequence their DNA. The closer two locations on the genome (*loci*) are together on DNA, the greater the chance that allelic forms of them are linked (will tend to be inherited together).

SNPs and other nucleotide polymorphisms are especially valuable markers for identifying function or behavior expressed by an individual because they can be easily, and accurately, detected. An allelic variant that can be easily detected and defined (score) is called a marker. The use of DNA polymorphisms as genetic markers has advantages over using phenotypic markers based on a trait of the organism. The main advantage is that DNA is all alike, and one can score, say, 5,000 different markers in two different individuals much more easily than one can determine the same number of individual genetically determined phenotypic traits (eye color, curly hair or straight hair, dimple in chin or no dimple).

Information on DNA polymorphisms for a population enables one to examine traits and outcomes in the population and determine which, if any, of those traits and outcomes are correlated with particular SNPs. Correlation is evidence that allelic variants of genes responsible for those traits and outcomes are located near the SNP.

Source: Brent, 2000.

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rapidly until it becomes cheaper to sequence an entire genome than to score a set of SNPs. Thus, comparisons of SNP differences among individuals, which are already being made on a large scale, will increase dramatically.

RNA Techniques

Genes are *transcribed* into messenger RNA (mRNA), and the different mRNAs are then *translated* into proteins, which do the work of the living organism. There is a general positive correlation between the identity and number of mRNAs present in a *sample* (an extract from a cell, tissue, organ, or organism) and the number of the encoded proteins present in that sample.

Unlike proteins, different mRNAs have similar chemical properties. Therefore, populations of them can be converted by identical manipulations into complementary DNA (cDNA), amplified (by PCR), and detected (by their ability to hybridize to oligonucletides or longer pieces of DNA, for example, immobilized on chips). The determination of the identity and number of different mRNAs in a sample is often referred to as *transcription profiling*, or *gene-expression monitoring* (GEM).

Many GEM methods can generate the same information. The best technology at present, Affymetrix photolithographic oligonucleotide arrays, would cost about $1,000 to measure 1,000 different mRNAs, or $1 per mRNA. Motorola and Corning have announced competing products that should cut this cost by a factor of 10. That cost will continue to drop rapidly, and it is fair to anticipate that in 10 or 15 years measuring the expression of as many human genes in a sample as one chooses, up to the entire set of 30,000 or so genes, will cost only a few dollars. The committee believes that widespread monitoring of gene expression using mRNA techniques will make it possible to gather data on human responses and, eventually, apply the information to situations in near real time.

Impact of Genomics on the Prevention and Treatment of Disease

Genomics will certainly alter the treatment and prevention of diseases (*pharmacogenomics*). Future therapies will depend on a complete understanding of the genetic biology of individuals. With genetic profiling, the effect of individual genetic elements on disease and fitness can be determined. Germ line sequences will be used to reveal inborn and somatic mutations that existed at birth or that have occurred over the life of an individual. Mutations that may predispose an individual to certain diseases, cancers, or environmental sensitivities will be identified. Many illnesses will come to be viewed as phenotypic manifestations of genetic differences.

Genomics-based treatment will become the norm. At the DNA level, there is already a wealth of information that can predict whether a person will respond well to a therapeutic technique. For example, people who carry certain allelic variants of cytochrome P450 CYP2D6 do not convert codeine into morphine and do not benefit from the drug; people with amplifications in CYP2D6 metabolize codeine so well that standard doses are ineffective (Sindrup and Brosen, 1995). Similar DNA-polymorphism-based stratifications of patients will help identify subgroups of military personnel that will benefit from, or be adversely affected by, particular drugs. At the RNA and protein level, expression monitoring will be developed to the point that changes in the patterns of expression of particular genes in patients with particular genetic makeups will provide early warnings of therapeutic or toxicological outcomes (e.g., after receipt of a vaccine or exposure to a chemical or biological threat agent).

Most of these genomic capabilities will be developed by the civilian sector for medical conditions and diseases that are of economic interest in the affluent countries of the industrialized world. Thus, the military will not always be able to rely on the market to generate capabilities for military applications. For example, if the military is interested in DNA, RNA, or protein information that can be used in chemical biological defense toxicology or in guiding malaria prophylaxis or therapy, it will have to complete that research itself.

The Army has good reason to take advantage of the knowledge genomics will provide and is in good position to further the state of the art. For example, the recent anthrax vaccination program undertaken by the Army was for a controlled population against an agent that is not addressed by general public health services. The Army was assigned to manage the administration of vaccinations to 2.4 million members of the military services and to monitor progress in each service (DOD, 1998). The immunization program consists of a series of six innoculations over an 18-month period, followed by an annual booster. The cost of vaccinating the total force over a six- to seven-year period was estimated to be about $130 million (USAF, 1998).

This and similar vaccination programs could provide a unique opportunity for use of genomic techniques to improve prophylaxis. Administration of a program on the scale of the anthrax program requires extensive monitoring and follow-up and could provide a perfect test environment for monitoring responses to vaccination in a large population. Advancing the understanding of the genetic basis of different responses to this vaccine would advance the prescription of drugs and vaccines by genotype.

Most vaccinations can cause adverse effects in small numbers of people receiving the vaccine. Anxiety about adverse effects to single vaccine (for example, those that occurred during the swine flu vaccination program in the 1970s) often lead to a general anxiety about all vaccinations. During the course of the anthrax program, a small number of service members refused to be inoculated.

Insofar as the military mirrors the larger society, such

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social concerns are an important factor in successful vaccination programs. The public discussion of the Gulf War syndrome revealed a widespead distrust of the protection provided to American service personnel shipped to strange lands and subjected to foreign risks. Whether justified or not, the anxieties are real. Anxieties about vaccination in both the military and the general population are likely to persist. A long-term approach to ameliorating this concern could include the systematic collection of genomic data to monitor the responses of participants in vaccination programs.

Assuming that issues relating to personal privacy can be overcome, the Army has a unique opportunity to collect and use genomic data. For example, if the Army collected genotypic information on individuals who receive a vaccine and compared that information with later information about the success of the immunization or adverse effects, it could determine if genetic markers could be correlated with the vaccine's ineffectiveness. Even weakly predictive information could be useful. For example, information that a given individual was not likely to be easily immunized by a vaccine could be used to recommend a different kind of prophylaxis, such as additional booster doses of the vaccine, increased clinical surveillance, or auxiliary prophylactic therapy, such as antibiotics in addition to the vaccination.

The Army is also in a position to add this information to its research base. It would be of great value to the Army, and possibly to society as a whole, to increase epidemiological monitoring of troops deployed abroad and to add genomic information to such studies. In collaboration with academic and industry researchers, the Army could contribute to a better understanding of vaccination and to the development of vaccines tailored to individual genotypes.

Prediction and Enhancement of Soldier Performance

Combat effectiveness can be increased by enhancing the performance of individual soldiers. Because genomics information offers clues to improving human performance, it could provide the Army with means of increasing combat effectiveness.

Prediction of Performance and Outcomes

Genomic techniques will enable the measurement of differences at the cellular level in DNA, mRNA, and expressed proteins. These measurements could potentially lead to predictions of differences in performance among soldiers, as well as the direction and tailoring of individual therapies and augmentations. In the future, genomic methods could be used to screen or supplement physical tests for qualities such as strength, endurance, marksmanship, or the ability to function when deprived of sleep; these predictions could be used to help in assigning individuals to perform appropriate tasks.

The Army has long used physical characteristics, such as vision or physical size, to subdivide soldier populations and has used the outcome of physical and mental tests to determine who is qualified to pilot helicoptors or to join elite combat units. Experience has shown that some individuals cope with the horrors of combat better than others, but the basis for these differences is not understood. Many police and fire departments use psychological screening tests to evaluate the ability of individuals to deal with stress. Army experts believe that psychological screening may be used to improve soldier performance in close-combat units (Rhem, 2000). Genomic methods may eventually be used to supplement these performance-based tests.

Intelligent decisions based on genomic information can only be made with a clear understanding of what biological information does and does not indicate. Predicting the response of an individual will always be highly incertain. Stratification, particularly when the outcomes can result in inclusion or exclusion, should be based on performance criteria rather than genomic criteria. If genomic criteria are used to supplement other selection tools, the criteria should have a mechanistic explanation whenever possible, be highly predictive of performance, and be continually reviewed and revised.

The use of genomic data to predict differences in performance among individuals requires a careful consideration of ethical issues. A good example is the sickle-cell trait, for which the underlying mechanism is well understood. The sickle-cell trait is caused by a point mutation difference (a SNP) in hemoglobin. The point mutation causes differences in the biophysics of the hemoglobin that, among other things, affect how it binds oxygen in low-oxygen environments. If someone is homozygous for the mutant hemoglobin, he or she will not do as well in low-oxygen environments. A DNA test that measures the presence of this mutation will be measuring the cause of the difference in oxygen utilization and will be highly predictive. Other SNPs linked to the sickle-cell trait will be less predictive. Here, the facts that the mechanism is well understood and that a direct test for the trait is available determine what constitutes a fair exclusionary criterion. Measuring for a less predictive SNP might result in the exclusion of qualified individuals.

Emerging genomics tools offer the Army an outstanding opportunity to improve the understanding of the biological bases of differences that affect military outcomes. Because of its mission orientation, the Army may also be in a unique position to benefit from genomic monitoring. In all cases, broad ethical issues, such as privacy and equal treatment, must be addressed, and other issues may arise as we learn more about ourselves than we wish to know or than we wish others to know. Assuming that these issues can be resolved, the committee believes the Army should use genomic monitoring tools to further its purposes.

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Enhancement

Physicians distinguish between therapy (restoring a function to normal) and enhancement (boosting a function above the norm) and have traditionally been uneasy with the ethics of enhancement (a good discussion can be found in Kramer, 1993). For the military, therapy, enhancement, and augmentation may all be desirable. As long as social norms of acceptable drug use are observed, the Army should welcome drugs that could ease the adjustment to another time zone or to longer periods without food or sleep; the Air Force should welcome a drug that could increase the G-force a pilot can endure before blacking out; and the Navy should welcome a drug that could ease motion sickness. To be acceptable, the drug technologies must be both safe and reversible. Guaranteeing soldiers that they will be able to return to their original physiological profile (excluding normal wear and tear) will be very important.

In general, performance-enhancing drugs are not likely to be a focus for development by the private sector. The pharmaceutical industry spends hundreds of millions of dollars to develop new drugs targeted against diseases in affluent countries. The markets for performance enhancers, including the military, are smaller, and it is not realistic to think that the industry, much less the military, will be able to spend large sums on development.

Fortunately, radically cheaper and faster approaches to drug discovery are emerging. These include the use of combined genomic information with modern chemistry to generate new drugs and the use of many kinds of genomic information to streamline testing in animals and humans. Therefore, if the military wishes to realize the promise of enhanced performance that advances in biology, genomics, and chemistry can provide, it will have to make common cause with the other constituencies trying to reduce the time and lower the cost of drug development.

Key Recommendations

The Army can take advantage of commercial improvements in gene-expression-monitoring techniques to monitor threats to soldiers, as mirrored via gene expression, in response to external stimuli and to provide new methods of improving soldier training and performance. The Army should optimize gene-expression-monitoring techniques for soldier applications, especially for the detection of target threat molecules through toxicological genomics.

In the next 15 years, static genomic (DNA) information will be used to target individuals and to direct therapies tailored to be safe and effective for their genotype and to suggest differences in performance. Dynamic genomic (mRNA and protein) information will be used to predict differences in performance, track changes in individuals, and provide early warning of positive and negative health events. Complex ethical and privacy issues will have to be addressed, but the committee believes that guidelines for use of genomics technologies will evolve as the benefits become more firmly established.

Research in genomics is an important way for the Army to ameliorate the risks associated with placing susceptible individuals in harm's way. The Edgewood Chemical Biological Center has in-house facilities that are uniquely situated and equipped to pursue meaningful genomics research. The Army should develop prophylactic tactics and protocols for rapidly detecting pathogenic or cytotoxic agents to which soldiers may be exposed and for which both high and low levels of exposure could have long-term consequences for their health and performance. The Army should monitor developments in genomics and take advantage of advances to improve its screening tools.

The Army should become an early user of genomic data. It should develop predictors of individualized immune responses to vaccines so that the vaccines can be tailored to genotypes. The Army should lead the way in laying groundwork for the open, disciplined use of genomic data to enhance soldiers' health and improve their performance on the battlefield.

TRENDS IN DRUG DEVELOPMENT

The advent of genomics, screening techniques based on a mechanistic understanding of molecular and cellular biology, the widespread use of structural biological information, and improvements in medicinal chemistry and computational chemistry have dramatically improved the processes leading to discovery and development of new therapeutic compounds. Biotechnology will be enormously prolific in the next 10 to 25 years. The following developments will impact the Army:

- cheaper means of discovering and testing new therapeutic compounds
- lower costs of manufacturing through improvements in conventional production methods and the adoption of new ones

Currently, the development of a new therapeutic compound requires 7 to 12 years and costs more than $400 million per product. Drug development is a complex, multistep process beginning with an investment in research leading to an understanding of the biochemistry and function of a putative compound (discovery research), followed by production of a small quantity of material, assessments of toxicity in cell and animal studies (preclinical studies), development of process-scale manufacturing methods, applications to regulatory authorities for permission to conduct clinical trials, clinical trials, and posttrial evaluation.

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Protein Therapeutic Compounds

Until the 1980s, the discovery and characterization of proteins (e.g., hormones, antibodies, derivatives of cell-surface receptors, and other protein molecules) as drugs was rare. Today, several dozen therapeutic protein compounds are approved for sale in the United States and Europe, and hundreds more are in development. These include hormones, cytokines, growth factors, monoclonal antibodies, and therapeutic enzymes; most of these compounds are derived from genetically engineered organisms. Protein therapeutic compounds have been effective against diseases as varied as diabetes (insulin), cystic fibrosis (DNAse), hemophilia (Factor VIII), some cancers (interleukin-2, α-interferon, humanized anti-Her2 antibody), and hematopoietic difficiencies (erythropoietin). Most of these compounds either replace or supplement proteins with known functions (e.g., erythropoietin, insulin, Factor VIII) or bind to and/or affect known, well-studied receptors or infectious agents (e.g., anti-Her2/Neu antibody).

Genomics has greatly facilitated the discovery of new protein therapeutic compounds. In addition to the conventional pharmaceutical industry, specialized companies, such as Human Genome Sciences, Millennium Pharmaceuticals, Amgen, Genentech, and Genetics Institute, are making wholesale use of genomic methods to identify potential therapeutic compounds. Of the thousands of human genes, 5,000 to 10,000 may encode secreted proteins, and a significant fraction of these probably have biological effects that could be useful in some therapy (e.g., B-lymphocyte stimulator [BlyS] and osteoprotegrin [Opg1]). Thus, although all secreted proteins may be discovered and characterized by 2015, the development of therapeutic applications will probably take longer.

Protein therapeutics is not restricted to secreted proteins. Most existing therapeutic compounds (both small-molecule and protein) act by enhancing or inhibiting the function of a cell-surface receptor; therefore, it is widely believed that novel cell-surface receptors represent a promising class of therapeutic targets. It is estimated that approximately 20,000 human genes encode cell-surface receptors. One of the most common ways of affecting a response through a cell-surface target is to use a monoclonal antibody that binds to the receptor (see Box 7-3).

Protein Expression and Production

The majority of existing protein therapeutic compounds and vaccines are derived from genetically engineered bacterial, yeast, or mammalian cells grown under well-defined conditions. The manufacturing methods involve careful purification to ensure that the protein is free of impurities. For human proteins that have large therapeutic effects from small amounts of protein, production is not the primary cost driver. For proteins that must be administered in large amounts, or over a period of many years, the cost of production is a major factor. Recently, lower cost production methods have been devised, including expression of transgenic proteins in the milk of goats and cows, in chicken eggs, and even in plants. The abundance of new proteins will accelerate trends in the commercial sector toward improving protein expression, characterization, and purification. The result will be to speed up and lower costs of the development of novel protein therapeutic compounds.

Small-Molecule Therapeutic Compounds

Small-molecule therapeutic compounds dominate the drug market. Although protein therapeutic compounds, such as monoclonal antibodies, represent a rapid route to safe and effective products against novel targets, they have several significant drawbacks. Most significantly, proteins are not generally available orally and must be administered by injection. This limits their therapeutic use to proteins directed against truly life-threatening conditions (e.g., to treat diabetes), for which daily injections are acceptable, or to treatment modalities in which less frequent injections can be beneficial (e.g., anti TNF-α to treat rheumatoid arthritis).

Hundreds of small-molecule therapeutic compounds (e.g., aspirin) derived from synthetic compounds and natural products are on the market. Most of these are designed to treat medical problems with significant markets in affluent countries, such as pain, inflammation, high blood pressure, blood clots, depression, schizophrenia, cancer, diabetes, and Alzheimer's disease. The Army does not and should not support research in these areas. However, the Army does need treatments for infectious diseases, such as malaria, that could have an adverse impact on military operations.

The Army should be very interested in developing small-molecule drugs for indications other than infectious diseases, such as drugs to ameliorate shock caused by blood loss. As sequencing and structural information on all proteins becomes available, the discovery and design of small-molecule compounds that interact with specific novel targets will improve. The pharmaceutical industry will continue to invest heavily in these developments in the next 10 years, and the Army should track these developments closely.

Countering Chemical and Biological Threats

As the executive agent for the DOD Chemical Biological Defense Program, the Army is responsible for the development and acquisition of therapeutic compounds that could be used to counteract the effects of biological and chemical warfare agents, a "market" area that is not likely to be addressed by the commercial sector. In-house expertise will be needed to take advantage of developments in genomics and other areas that might enable leveraging of limited resources to produce vaccines, identify protein therapeutic compounds, identify targets for small-molecule drugs, and

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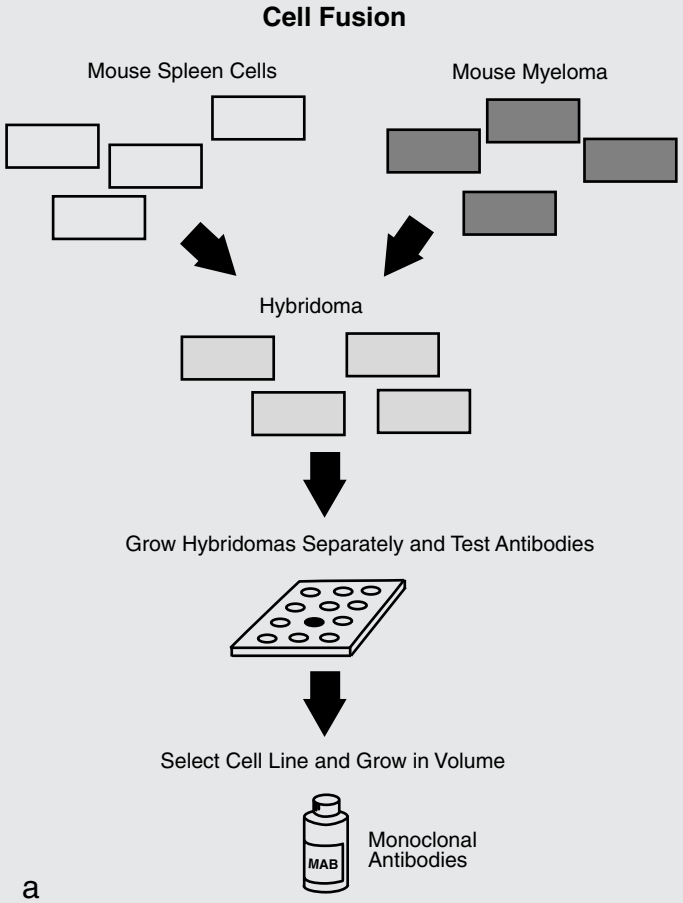
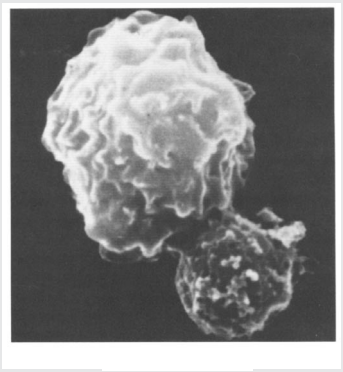
BOX 7-3
Monoclonal Antibodies

In 1975, Georges Kohler and Cesar Milstein fused cells derived from mouse B lymphocytes, which secrete antibodies, to mouse myeloma tumor cells, which can grow indefinitely in culture (a, at right). Fused cells, called *hybrid myelomas* or *hybridomas* (b, below), can grow in cell culture and produce large quantities of antibodies. Populations of cells derived from a single founder cell are clonal; therefore, the chemically identical antibodies they secrete are called *monoclonals.* Monoclonal antibodies are useful diagnostic agents and, increasingly, protein-based drugs. Unfortunately, humans mount an immune response to monoclonal antibodies from mice, known as the human antimouse antibody (HAMA) response; therefore, current practice is to generate "human" monoclonal antibodies. One way to do this is by "humanizing" murine monoclonals by mutating the amino acids in the framework that differ between mice and humans. Another way is to derive monoclonal antibodies from the spleens of transgenic mice whose immune systems produce human antibodies. A number of *in vitro* "display" methods can be used to create large libraries of human antibodies or antibody fragments that can be screened against a wide range of targets.

Source: Olson, 1986.



a



b

otherwise accelerate the development of drugs. For example, a reasonable step would be for the Army to assemble a database of target molecules and make this database available to academic and commercial laboratories so that, if they chose, they could identify those compounds. Given the rapid developments in chemistry and computational structural biology, it may become possible in this decade for the Army itself to identify the most important compounds in some cases.

Closer interaction with the commercial sector might enable the Army to make better use of existing compounds. For example, an antiviral compound that was not developed

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by the commercial sector to treat flu symptoms because of the potential side effects might merit evaluation by the Army for conteracting pathogens on the battlefield. Moreover, there are now more than 10 immunomodulatory cytokines (e.g., interleukins, such as IL-1 through IL-18) approved for use in humans. In principle, the proper combinations of these would offer new means of modulating immune responses to existing or new infectious diseases. The Army will be the main driver for clinical research on diseases of interest mostly to the military.

Finally, the Army should also monitor developments in commercial production. Through outsourcing, the United States may become dependent on other countries for the production of many critical pharmaceutical intermediates. U.S. advantages in a conflict could easily be compromised if the Army must depend on sources from countries with which the United States is not allied for the production of critical materials. For example, the committee is aware of only one remaining facility in North America that produces intermediates for beta-lactam antibiotics. Although it probably does not make sense for the Army to invest in infrastructure for the production of these compounds, it would be logical for the Army to establish a database of facilities and associated capabilities available to use in case of an emergency. This database could also be extended to include other key upstream and downstream aspects of the pharmaceutical industry, such as the status of clinical trials.

Examples of Army-Industry Cooperation

Recently, DOD was able to leverage commercial development by gaining FDA approval to use ciprofloxacin (CIPRO®) to treat people exposed to aerosolized anthrax (*Bacillus anthracis*). This approval was based on safety and efficacy data from a DOD animal study. Bayer, the manufacturer, applied for the approval after extensive discussions with DOD, CDC, and the FDA (Inglesby et al., 1999). Without treatment, anthrax is 99 percent lethal to unprotected people (DOD, 1998). Although penicillin and doxycycline have been approved to treat anthrax, there are reports that strains of the bacteria have been engineered to resist these antibiotics. A Working Group on Civilian Biodefense, comprised of 21 representatives of academic, government, public health, military, and emergency management organizations, including the U.S. Army Medical Research Institute of Infectious Diseases, was organized through the Johns Hopkins Center for Civilian Biodefense Studies. The group recommended early antibiotic treatment with ciprofloxacin or other fluoroquinolone therapy as the first line of treatment, and the FDA approved its use in this circumstance.

Cooperation has come to be expected in the medical community but is less common in Army relationships with the commercial high-technology industry. The Army could take advantage of commercial research and development through cooperative research and development agreements (CRADAs), which would provide the Army with some of the benefits of teaming with industry. But CRADAs cannot provide industry with the same guarantees of future business as traditional teaming arrangements within the industry.

The DARPA Unconventional Pathogens Countermeasures (UPC) Program is an example of government funding for applications of interest to the military that has resulted in potentially fruitful research. Organizations funded by DOD for this program are using state-of-the-art or revolutionary technologies to address chemical-biological defense requirements. Like past DARPA projects, this research may also have great commercial potential.

Many of the future biotechnology applications of interest to the Army in the fields of genomics and drug discovery are outgrowths of biomedical research sponsored by another government agency, the National Institutes of Health (NIH). The Army should work closely with the NIH to build on the existing research base and should consider cosponsoring research, both to leverage NIH relationships with industry and to ensure that Army needs are met. The Army is currently cosponsoring nonmedical research with DARPA on the Future Combat Systems Program, and similar arrangements with other government agencies, such as NASA or the National Science Foundation, should be investigated.

The examples of successful government-industry-academic collaborations above underscore the potential gains to the Army that can be realized from close interactions with the biotechnology industry. In addition to the DARPA UPC model and the traditional, direct-contract model (e.g., contracts to produce doses of vaccine), intermediate arrangements, such as CRADAs for joint research, would be effective in areas where private sector investment is driving research in new and emerging areas.

The following mechanisms might also be used to leverage private-sector technology developments:

- contracts that allow participants to use commercial practices and retain intellectual property
- government funding to mitigate the technical risk of producing selected prototypes
- teaming relationships, which often lead to cooperative agreements between participants

These mechanisms can allay industry's reservations about working with government, including noncommercial accounting requirements, potential government audits, special regulations governing government contracts, restrictions on trade, infringements on intellectual property rights, the risk of procurement violations, or possible negative press if the biotechnology is perceived to be used for weapons.

Improved Technologies for Drug Delivery

With micro/nanofabrication technologies, devices and components could be manufactured from a constantly expanding array of materials with unprecedented precision (see

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section in Chapter 6 on miniaturization of biological devices). Considerable attention has been focused on using these technologies for the development of novel, advantageous methods of delivering therapeutic and, possibly, performance-enhancing drugs (NIH BECON, 2000).

Several different types of nanotechnology drug-delivery systems are under investigation to introduce drugs via implanted devices and by precise intravascular injection. Research is also being conducted on micromachined needle arrays for the delivery of drugs through the skin (Brazzle et al., 2000). This technology is expected to lead to significant progress in the transdermal delivery of proteins and small molecules. Lin et al. (1998) have proposed portable systems for reconstituting and delivering drugs on the battlefield.

Implantable microsystems for the controlled release of biotechnological molecules are also under development (Desai et al., 1999). The drug-delivery chip presented by Robert Langer and associates at MIT (Santini et al., 1999) can deliver multiple drugs in a pulsatile, remotely controlled or automatically regulated manner (Figure 7-1). This chip could be used to deliver insulin and health-promoting drugs, as well as performance-enhancing drugs or threat-mitigating drugs on demand.

Several approaches to the delivery of therapeutic molecules have led to currently available pharmaceutical products. These include biodegradable particles, liposomes, and the direct conjugation to immunological molecules. To overcome the limitations of these approaches, several groups are engaged in developing the microtechnological and nanotechnological foundations of intravascular delivery mechanisms (Nashat et al., 1998).

Trends and Barriers

Improved transdermal delivery and implantable, controlled-release microtechnologies are expected to be ready for commercialization in the short term. Implantable devices capable of self-activation through a feedback loop combining sensing and the release of a therapeutic agent could be available in 5 to 10 years. These devices will require the

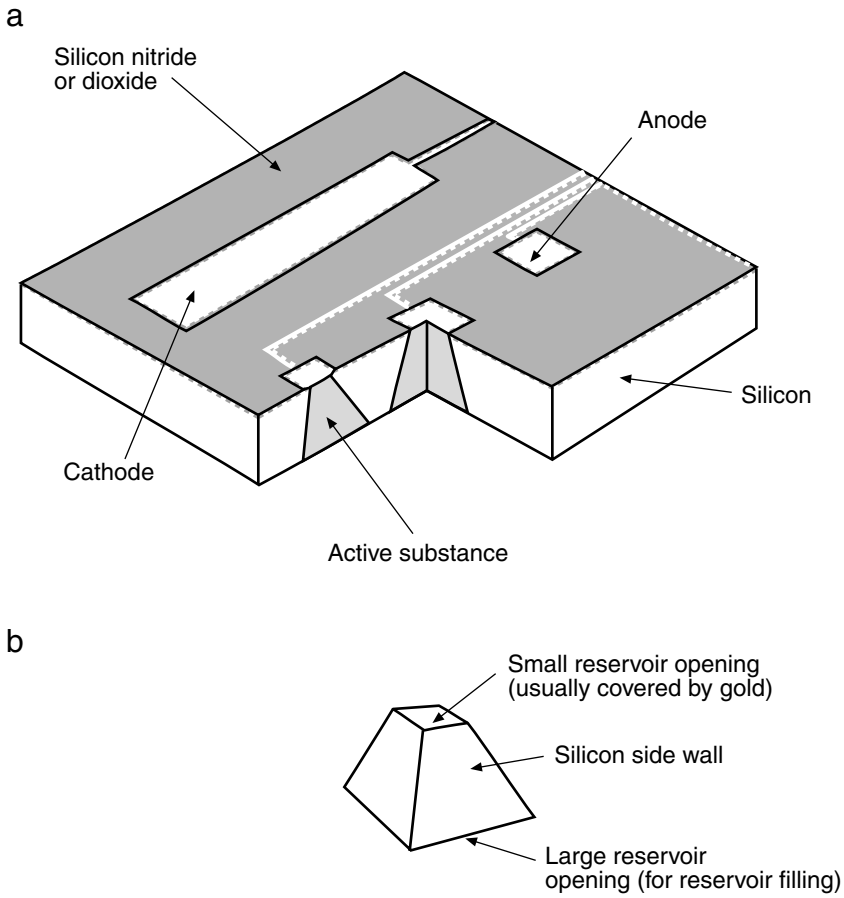

FIGURE 7-1 Drug-delivery chip.

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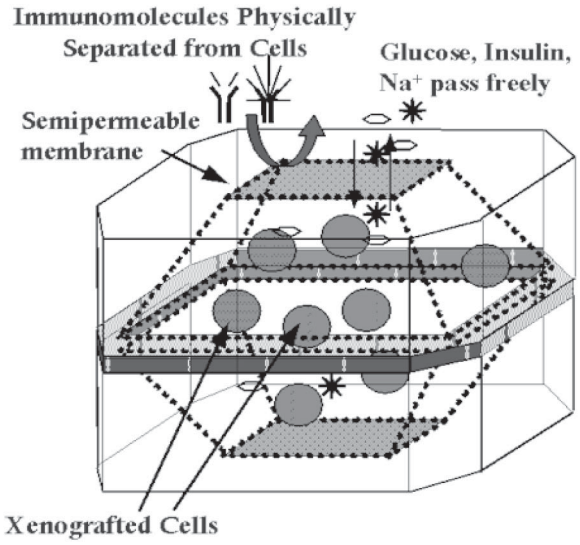

FIGURE 7-2 Cell-transplant biocapsule.

Source: Reprinted with permission from Desai et al., 1999. Copyright 1999, Kluwer Academic Publishers.

development of sensing and drug-release mechanisms and the technology to integrate them.

Extraordinarily difficult biological barriers will have to be overcome for the development of effective, biologically targeted, intravascular drug-delivery systems. Micro/nanofabrication scientists and life scientists will have to work together in the development of delivery technologies based on biologically inspired methods.

The high-throughput screening of molecules that are candidates for therapeutic drugs may be accomplished through new nanotechnological concepts as well as through other procedures. Micro/nanomachined templates might be used for the realization of cell culture environments that mimic the complexity of the biological environment (Bhatia, 1999). In addition, cell microenvironments may be instrumented to provide real-time measures of cellular responses to physical or chemical stimuli. These concepts may also provide the foundations for tissue-engineering constructs.

Technologies for Invasive Interfaces

Invasive interfaces (i.e., implanting a material in the body) may be necessary for sampling and analyzing molecules in the body. Micro/nanofabrication techniques are being used to develop a rejection-free method of transplanting biological cells for therapeutic use (Desai et al., 1999). Therapeutic drugs for insulin-dependent diabetes, chemotherapeutics, and local analgesics are driving commercial investments. The development of compatible biomaterials will also be a necessary part of the solution.

A recently developed microtechnological biocapsule (Figure 7-2) has been developed that is more efficient than previous methods of isolating the therapeutic from immune system response (immunoisolation), is more biologically benign, and is nondegradable. This technology may have particular specialized applications for the Army. For example, biocapsules could be used to implant cells that have been genetically altered to produce particular molecules. In this way, the biocapsule would become an implantable drug factory that could deliver performance-enhancing or therapeutic molecules for desired time periods.

The concurrent development of a fundamental understanding of cellular biology may interface with, and eventually supplant, such technology platforms, although it is difficult to predict exactly how. In their own way, cells may be considered to perform as biocapsules, and it may prove to be easier to reimplant an individual's own cells, or work with engineered cells, to achieve the same goals.

The nanopores in the cell transplantation biocapsule shown in Figure 7-2 are obtained by a combination of photolithography and sacrificial-layer technologies. Nanopore technology may also prove to be instrumental in the development of breakthrough instrumentation, such as ultrarapid sequencers. For other biological and nanotechnological constructs, however, much more work will have to be done.

Scalable fabrication methods will be necessary for commercial viability; without them, the private sector is not likely to invest energetically in the type of nanotechnologies described here. Private-sector interest in nonbiological nanotechologies (e.g., nanometer-sized holes, carbon nanotubes) seems certain. But advances in biological nanotech-

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nology (e.g., programmable molecular assemblers that may be necessary for implants) may not even be possible.

Basic research in nanoscience is now being funded by the Army as one of its Strategic Research Objectives (DA, 1998). The committee suggests that the Army adjust its focus on nanoscience to include the interfacing of biology with materials science to influence the direction of developments in biological nanotechnology. Lack of nanotechnology (with a biological component) is a barrier to important developments in sensor capabilities and can only be addressed through a broad-based effort by industry, government, and the military.

Implants and Biocompatibility

Protective packaging of an implantable sensor and associated actuators may or may not be possible because the sensor will have to be in direct contact with the biological milieu, which may generate adverse reactions from the body and lead to the rapid deterioration of the sensor, or both. Such complications have stalled the development of implantable glucose sensors to monitor therapy for diabetics for many years.

Like other biological material developments, current research to enhance the biocompatibility of silicon is focused on surface modifications that minimize unwanted, nonspecific protein adsorption. Leaders in the field include Frances Ligler and Bruce Gaber at the Naval Research Laboratory, Micronics, Inc. (a Seattle-based company), and Miqin Zhang at the University of Washington (Zhang et al., 1998). All of these researchers are attempting to covalently immobilize protein-resistant groups (e.g., alcohols, polyethylene glycol) along the principles developed by Mrksich and Whitesides (1996). Engineering protein adsorption on surfaces is crucial not only for *in vivo* implantable devices, but also for microfluidics and nanofluidics in laboratory devices. For instance, nonspecific protein adsorption on silicon-oxide islands spontaneously formed on silicon surfaces may foul microfluidic and nanofluidic devices. Recent developments in the field are discussed in Desai et al. (1999).

Implant materials may interact negatively with the biological milieu in a number of ways that collectively define the notion of biocompatibility. These include chemical or mechanical instability, release of harmful degradation products, cell toxicity, inflammatory reactions, formation of thrombi and emboli upon contact with blood, and the introduction of potentially carcinogenic alterations in nucleic acids. Drug-delivery implants may also fail if the effective release of the therapeutic payload is compromised by the scar tissue naturally produced by the recipient organism as a reaction to both the implantation surgery and the implant material.

Protein-implant and cell-implant interactions are determinants in these failure modes. For instance, the adsorption of plasma proteins is the priming event for the involvement of platelets and the clotting cascade that leads to the formation of thrombi and emboli. The activity of phagocytic cells at the implant site is believed to trigger a chain of events that leads to the formation of a fibrotic capsule around the implant. Ensuring biocompatibility is frequently a matter of engineering protein and cell resistance at implant interfaces, either directly on the implant material or indirectly by a biocompatible coating on the implant structure.

Biocompatibility is such a serious problem with semiconducting materials that direct contact between semiconductors and the biological milieu is not even mentioned in comprehensive references (e.g., Black, 1999; Horbett and Brash, 1995), even though semiconductors comprise the core technology of electrical stimulation devices such as pacemakers, wearable defibrillators, and pain-control and seizure-control implants. In these devices, the semiconducting materials are not directly exposed to the biological environment but are contained in a silicone-lined casing.

Solving the problem of biocompatibility, which is now considered a collection of difficult engineering problems, has been slow. A possible solution might eventually be found with the "biological" engineering of cells, but new ground will have to be broken in this field.

Biocompatibility issues will have to be overcome to enable the development of implant devices, such as biocapsules, to monitor soldier health or deliver antidotes to toxic agents. Such devices could increase soldier survivability, as well as unit combat effectiveness. The challenge for micro/nanofabrication technologists and life-science experts will be to collaborate on the development of biologically inspired methods and materials to improve drug-delivery technologies. The Army should monitor progress in implant research, drug-delivery technologies, and alternatives that could meet its needs.

Somatic Gene Therapy As an Alternative to Implanted Devices

By 2025, it is likely that somatic gene therapy will be developed to the point that it can be used to direct the synthesis of protein therapeutics in individual soldiers, thus obviating the need for implantable devices. For example, gene therapy agents could be transfected into cells by bombarding a patch of skin with DNA-coated pellets from a gene gun. As the cells are sloughed off, expression of the therapeutic protein would naturally cease but could be renewed by another application of the agent. By 2025, reliable and robust means of delivering DNA constructions to other cell types will also become available. In fact, much or all of the technology implanted into the individual soldier will probably be derived from the individual's own cells rather than from fabricated devices.

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Barriers to Development of Therapeutics and Vaccines

The use of genomic information to improve the protection of military personnel, and even to help direct their tasks and training, will require that the Army educate itself about the limitations and potentials of these techniques and, in some cases, will require that complex ethical issues be addressed. In addition, the fabrication of devices that can come into contact with the interior of the body also faces formidable technical challenges. Controlled delivery of therapeutic molecules might be possible someday by "devices" that do not have fabricated components but consist solely of engineered cells reimplanted in the body or even self-assembled after injection. For the present, however, the Army can address the barriers to immunization and drug development using proven developments in genomics and biotechnology.

Immunization Barriers

The challenges facing the Army's research on vaccines should be considered in the context of the larger society. During the many decades the Army has been engaged in the development of vaccines, it has developed close working relationships with industry and has made substantial progress. The Army has the expertise and experience to define and address its needs for the development of vaccines. In fact, in some cases, the Army is the world authority. The Army also understands commercial-sector strengths and weaknesses and has worked well with industry.

Currently, the Army is actively involved in the development of vaccines against malaria (*Plasmodium vivax*), diarrheal diseases (including *Rotavirus*), flavivirus (including dengue), and rickettsia and is actively pursuing research on hemorrrhagic fever viruses (e.g., filoviruses like Ebola) and other highly lethal viruses (Hoke, 2000). The Army also supports significant programs in the development of adjuvants (see Appendix D). In general, the Army has tried to cooperate with, and coordinate its research with, commercial partners. But the interests of commercial companies, whose research budgets are much larger than the Army's, are not necessarily compatible with the Army's objectives. Most of the barriers to the development of vaccines of interest to the Army can be attributed to these business realities.

The global vaccine industry is dominated by six big companies, all of which also make therapeutics. The companies are Merck, Smith-Kline Beecham (soon to become GlaxoSmithKline), Wyeth-Ayerst (now part of American Home Products), Pasteur Merrieux (once part of Rhone-Poulenc Roher, which is now part of Aventis), Bristol-Myers Squibb, and a relative newcomer, Chiron (a biotechnology company in the process of transforming itself into a fully integrated vaccine and pharmaceutical company).

For these big companies, research and development costs for a single vaccine can easily top $200 million, and the time from program start to marketing may be as long as 12 years. To counter these costs, a potential vaccine must have peak revenues of at least $200 million per year and must earn revenue for 10 years. Therefore, the target price for a single dose or course of vaccinations is more than $50, and usually more than $100. These economic realities all but preclude commercial investments in vaccines of interest to the Army.

Although a number of small companies are working on vaccines, they usually try to form corporate partnerships with one of the larger companies, which supplies funding. The larger partner usually has exchange rights to market the successful product. Barriers to the entry of new firms in the vaccine business include the vast specialized expertise required to conduct human testing, market the product, and defend the product against product liability claims.

Liability claims have reinforced the conservative practices of the vaccine industry. In a small number of people, vaccinations have adverse effects. The best example of this is the swine flu vaccination program in 1976. In February of that year, the CDC confirmed that an influenza outbreak at Fort Dix had been caused by the swine-type influenza A virus. Subsequently, the Department of Health, Education, and Welfare, concerned about a major flu epidemic similar to the epidemic in 1918, recommended that the federal government vaccinate all Americans, and more than 40 million people were vaccinated. However, the program was suspended following reports that people in more than 10 states had developed of Guillain-Barré syndrome (GBS). By January 1977, more than 500 cases and 25 deaths had been reported (Langmuir, 1979). Despite the lack of a definitive biological explanation for the association of the swine flu vaccine and GBS, there was strong evidence of a causal relationship, which led to millions of dollars in lawsuits (Laitin and Pelletier, 1997). The result of this incident was a decrease in public confidence in vaccination programs and a dampening of the enthusiasm of the pharmaceutical industry to develop vaccines.

In general, because of concerns about safety and product liability, the technology to create new vaccines has not evolved as rapidly as the underlying science. The impact of this conservatism has been a gap between new biological knowledge and capabilities and the availability of new, cost-effective vaccines. Recombinant subunit vaccines coming onto the market are based on 1970s technology. The regulatory, liability, and testing environment has inhibited the development of vaccine technology to the point that current knowledge in genomics has had little impact on the development and production of vaccines.

Drug Development Barriers

Given the current state of drug discovery and development, the time and cost of producing a new drug could potentially be greatly reduced. Genomic technologies could be used to reveal targets; high-throughput screening or compu-

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tational structural biology and chemical informatics could suggest inhibitors; a combination of computerization and mechanization could improve compounds; the collection of genomic information from animal studies, including new transgenic animal models, could accelerate preclinical development and toxicity studies; and genomic information could allow much smaller targets and, perhaps, single-step human clinical trials with near-real-time monitoring of beneficial and adverse events.

The pharmaceutical industry is subject to some of the same business pressures and liability problems as the vaccine industry, although the number of companies involved in the oligopoly is larger and the industry as a whole is immensely profitable. Nevertheless, radically new ideas are likely to depend on the entry of new firms into the market. The Army has very little leverage over the business landscape but can, by targeted spending of its own research funds and by working with other government and regulatory agencies, encourage new companies that will make use of the new technologies, thus creating an environment more responsive to Army concerns.

Key Recommendations

The Army should take personal interest in developing small-molecule drugs to ameliorate shock caused by blood loss. As sequencing and structural information on all proteins becomes available, the discovery and design of small-molecule compounds that interact with specific novel targets will improve, and the Army should track these developments closely.

The Army, and the country as a whole, are becoming increasingly dependent on foreign sources for many critical therapeutic materials. Even though it may be prohibitive for the Army to invest in manufacturing infrastructure, the Army should develop and maintain a database of global manufacturing capabilities, including the biology, processes, and equipment to produce critical therapeutic materials. This database should include other key upstream and downstream aspects of the pharmaceutical industry, such as the status of clinical trials.

Although federal and state regulation of research and development in therapeutics affects both military and civilian products, in exceptional circumstances, national defense needs should be given special priority and should be provided a legal basis for taking action. In urgent cases, the Army simply cannot wait while developers strive to meet the extremely high (> 99.99 percent) effectiveness demanded by federal regulators and civilian consumers. Delays could be critical if the development of antidotes, quasimedical devices, and other biotechnology products are needed to meet immediate military requirements.

The government should define and certify special processes for the development and approval of biotechnology applications to meet exceptional Army and other defense needs. DOD must have the ability to identify exceptional requirements and expedite the development of products with the potential to benefit soldiers confronted with an urgent threat or special need.

Developments in cell biology, immunology, molecular genetics, and genomics have led to new concepts that could greatly improve the safety and efficacy of vaccines and reduce the time and lower the cost of vaccine development and production. As the pace of genomics advances quickens, the Army will be hard pressed to take advantage of the many opportunities to provide better vaccines. Reducing the time involved in clinical trials should be a high priority.

The Army should build on its strengths in vaccine development and fund new technological approaches, including genomics developments, DNA vaccines, cell-based vaccines, and monoclonal antibodies. It should also explore using transgenics to shorten the clinical-trial phase for defining toxicity and using pharmacogenomics to shorten the time for Phase III clinical trials, which involve large populations and are difficult, expensive, and prolonged.

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8

Conclusions and Recommendations

This chapter presents the committee's conclusions and recommendations. It begins with a discussion of overarching issues, continues with specific recommendations for Army investment, and concludes with other findings on topics encountered during the study. The committee considered opportunities afforded by biotechnology in application categories relevant to soldier operations in the present and foreseeable future through 2025. In keeping with national policy, the study did not consider offensive biological weapons; however, the committee believes that all biotechnology development should be undertaken with defenses against such weapons in mind.

As requested in the Statement of Task, the committee considered technologies likely to be important to the Army in the next 25 years. As a framework for its evaluation of prospective and applicable biotechnologies, the committee developed a list of enduring Army applications, with topics listed in alphabetical order (Table 8-1).

BIOTECHNOLOGY DEVELOPMENT AREAS

The committee evaluated biotechnology developments in five broad application categories: sensors; electronics and computing; materials; logistics; and therapeutics. The following areas in each category were then identified as providing significant opportunities for the Army:

- sensors: assay analysis; detection methods; chip architectures
- electronics and computing: protein-based devices; biocomputing; biomolecular hybrid devices
- materials: tissue engineering; biologically inspired materials and processes; hybrid materials
- logistics: miniaturization of biological devices; functional foods; biological energy sources; renewable resources
- therapeutics: genomics and proteomics; drugs and vaccines; drug delivery systems

OVERARCHING CONCLUSIONS

The biotechnology industry now surpasses the aerospace industry in market capitalization, research expenditures, and complexity, and the research and development (R&D) budgets of the large pharmaceutical companies dwarf the Army's R&D budget. Unlike traditional defense developers, commercial developers in biotechnology are "discovery-oriented"; that is, they are pursuing developments in many directions as determined by the marketplace, which so far is predominantly medical. The Army, however, has become used to managing and influencing R&D directed toward specific procurement objectives.

Conclusion 1. To keep pace with the unprecedented rate of discovery and the anticipated increase in biotechnology developments, the Army will have to establish new, effective partnerships with the emerging biotechnology industry, participate in research, leverage research and developments in the commercial sector, and develop its internal capabilities (organization and personnel) to act on opportunities as they arise.

The biotechnology industry is much less dependent on the military for its existence than other industries with which the Army and other services have routinely interacted. Therefore, the Army will have to use different mechanisms for involving industry in meeting Army needs.

Realizing new applications for biotechnology in a nonmedical area—armor, for example—will require the application of biological disciplines to areas outside traditional medical technology. Although most Army medical applications are similar to (sometimes identical to) civilian applications, nonmedical applications will be much more difficult to identify and influence.

Recommendation 1. The Army should adopt new approaches toward commercial developers to accommodate

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TABLE 8-1 Prospective Army Applications

Application	Description
Camouflage and concealment	Biomaterials with stealth characteristics; nonilluminating paints and coatings.
Combat identification	Biological markers to distinguish friendly soldiers.
Computing	DNA computers to solve special problems; biologic models to suggest computer algorithms.
Data fusion	Associative memory and other protein-based devices; artificial intelligence.
Functional foods	Additives to improve nutrition, enhance digestion, improve storage characteristics, enable battlefield identification, reduce detectability; edible vaccines; fast-growing plants.
Health monitoring	Devices to provide feedback on soldier status, enable remote triage, and augment network of external sensors to provide intelligence on chemical, biological, or environmental agents.
High-capacity data storage	Rugged computer memories for individual soldiers.
High-resolution imaging	High-resolution alternatives to semiconductor imagers.
Lightweight armor	Protection for soldiers and combat systems; systems with living characteristics, such as self-repairing body armor.
Novel materials	Biologically inspired materials; biodegradable consumables; genetically engineered proteins; renewable resources.
Performance enhancement	Cortical implants; computer input and display interfaces; prostheses control; sensory enhancement; antidotal implants; gene-expression monitoring; performance-enhancing drugs.
Radiation-resistant electronics	Protein-based components; biomolecular hybrid devices; biomolecular diodes; bio-FETs (field effect transistors).
Reductions in size and weight	Cell-based processes; molecular electronics; biochips; nanotechnology.
Sensing battlefield environments	Laboratories-on-a-chip to detect and identify chemical, biological, and environmental threat molecules on the battlefield; coupling of diagnostic and therapeutic functions.
Sensor networks	Remote sensors mounted on vehicles and carried by soldiers to augment threat intelligence.
Soldier therapeutics	Drugs to counteract shock; genomics-based, directed therapies; optimized responsiveness to vaccines.
Soldier-portable power	Biological photovoltaics; cell-based energy systems.
Target recognition	Protein-based devices for pattern recognition; artificial intelligence.
Vaccine development	Reduced development and production times for small-scale requirements to respond to diseases encountered in exotic locales.
Wound healing	Engineered skin, tissue, and organs; wound dressings and treatments to curtail bleeding and accelerate healing.

cultural differences between the government and the biotechnology industry.

Mechanisms that would encourage fruitful relationships between government and industry include contracts that allow businesses to use regular business practices and protect intellectual property rights for nongovernment applications; government funding to mitigate the technical risks of producing prototypes; and minimal requirements for noncommercial, government accounting and audits. These measures would alleviate some of industry's reservations about government contract regulations, restrictions on trade, and the possible negative perception of working with the military on "biological things."

In addition to working relationships with companies, the Army will have to form novel relationships with small and large industry organizations and other government agencies with the same or similar interests. Essential government partners include the National Institutes of Health (NIH), Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC). Making the most of these new relationships will require that the Army develop and maintain its own expertise in bioscience and bioengineering, both to contribute to and gain insights from the biotechnology community and to build on existing expertise and established relationships between the Army medical community and industry.

Conclusion 2. Although medical applications are not the focus of the present study, the commercial markets for medical applications will determine the direction of developments in biotechnology in both medical and nonmedical categories. Engineers and scientists will necessarily become experts in areas that extend biology to other disciplines. To influence developments in Army-significant, nonmedical areas, Army personnel will have to expand their understanding of the role of biology.

Future developments in biotechnology will be accomplished by groups of engineers and biological and physical

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scientists working together. To leverage discoveries and developments with the highest likelihood of payoff, the Army will need sophisticated in-house expertise in biologic disciplines related to genomics, drug discovery, biosensors, biomaterials, and other specialized areas. Monitoring commercial developments will require broader expertise than is normally required to conduct research. To develop and maintain the needed range of biotechnology expertise, the Army will require both a pool of educated personnel and a strong, in-house experimental program.

Recommendation 2a. To operate effectively in the multidisciplinary environment of future biosystem development, the Army will have to invest in education. In addition to its existing expertise in medical research and development, the Army will need a cadre of science and technology professionals capable of translating advances in the biosciences into engineering practice.

Ideally, these professionals will serve in a mix of permanent and rotating positions. The permanent positions would ensure full-time expertise and continuity of focus on biological developments outside the Army. The rotating positions would enable the Army to interact with key segments of the biotechnology industry and would ensure that the Army remains involved in the latest commercial developments. In short, this cadre of experts would monitor developments, enable the Army to identify new opportunities, publicize Army requirements, evaluate alternative biotechnologies, and otherwise influence the course of developments beyond traditional medical applications to future nonmedical applications.

Recommendation 2b. The Army should conduct a study that focuses on future biomedical applications. The study should explore biological implants, biocompatibility, medical biomaterials, medical defenses against chemical and biological agents, and pharmacogenomics. These will have far-reaching implications for future military operations but were outside the range of expertise represented on the study committee.

PRIORITIES FOR RESEARCH

The opportunities in biotechnology discussed in this report are summarized in Table 8-2. Each item includes the committee's recommended investment priority, estimated time frame for realization (i.e., midterm [5 to15 years] or far term [15 to 25 years]), and level of commercial interest. The Army should be especially vigilant in monitoring technologies with high commercial interest in anticipation of industry developments that might be leveraged to meet the Army's needs.

The committee recommended an investment priority of high, medium, or low for each biotechnology area covered by the study. Army investments in research can be catalytic and serve the purposes of both the Army and society as a whole. Commercial technology developments may also go a long way toward addressing Army needs. The committee recommended a "high" investment priority if the technology applications are likely to fill a perceived void for the Army on future battlefields, if the biotechnology appears to offer the most promising avenue toward solving an Army problem, and if the biotechnology is not likely to be developed by industry. A "medium" investment priority was recommended in areas where an Army-sponsored research activity can be used to help open windows on commercial developments; such activities might be conducted as in-house basic research or in the context of cooperative agreements with academia or industry. A "low" priority for investment was assigned to biotechnology areas that should be monitored by an Army expert but do not appear at this time to justify research funding.

Conclusion 3. Five biotechnology areas meet the criteria for high-priority Army investment. These biotechnologies are highly likely to support applications for predicted, Army-unique mission requirements in the next 25 years. In addition, the committee identified four other areas with significant military potential in which focused research investments would help to surmount barriers to developments.

Recommendation 3a. The Army should focus its research in the following high-priority areas in which developments are likely to be accelerated by Army investment:

- three-dimensional (volumetric) memory for rugged data storage
- self-replicating systems for wound healing
- small-scale vaccine production
- shock therapeutics
- vaccine stratification by genomics and toxicogenomics

Recommendation 3b. The Army should support basic research in the following areas to overcome barriers to development:

- determination of target threat molecules for sensors
- proteins for radiation-resistant electronics
- hierarchical design models for bioinspired materials
- structural interfaces for device substructures

Conclusion 4. Most of the biotechnology areas with high potential for the Army are subjects of ongoing research and development by government and/or industry. Continued research in these areas is highly likely to result in near-term advances that will be important for future Army applications. Regardless of the priority assigned by the committee, a biotechnology area may still be important to the Army because opportunities arising from advances in the fundamental biosciences may appear on the horizon with little or no

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TABLE 8-2 Biotechnology Development Areas

Development Area	Biotechnology	Investment Priority	Time Frame	Commercial Interest
Assay Analysis	Microfabrication/microfluidics	medium	midterm	high
	Affinity reagents	medium	midterm	high
Detection Methods	Optical detectors	low	midterm	high
	Detector arrays of affinity molecules; DNA chips; protein chips	medium	midterm	high
Protein-Based Devices	Optical-holographic high-density memories	low	midterm	medium
	Three-dimensional volumetric memories	high	midterm	low
	Associative memories and processors	medium	midterm	medium
	Artificial retinas	low	midterm	low
	Pattern-recognition systems	medium	midterm	low
	Spatial light modulators	low	near term	low
Biocomputing	Biological models	medium	far term	low
	DNA computers	low	far term	low
Biomolecular Hybrid Devices	DNA-based optical-signal processing	low	midterm	medium
	Biomolecular diodes	low	midterm	low
Tissue Engineering	Cartilage repair and replacement	medium	midterm	high
	Neural bridging	low	far term	medium
	Self-replicating systems	high	far term	medium
	Stem cells	medium	far term	high
	Synthetic biomaterials	low	far term	medium
	Portable, artificial, assisting devices	low	far term	high
Bioinspired and Hybrid Materials	Biologically produced materials	medium	far term	medium
	Biomineralization: organic/inorganic nanocomposites	low	far term	medium
	Hierarchical systems; biocomposites	medium	midterm	medium
Miniaturization Technologies	Microreaction technologies	low	midterm	high
	MEMS-based microfluidic systems	medium	midterm	high
	Biochip architectures	low	midterm	high
	Biological nanotechnology	medium	far term	high
Functional Foods	Genetically engineered foods	low	near term	high
	Edible vaccines	medium	midterm	medium
Biological Sources of Energy	Biological photovoltaics	medium	midterm	medium
Renewable Resources	Renewable fuels	medium	midterm	high
	Nonmedical specialty products based on engineered organisms	low	near term	medium
	Ecological life-support systems	low	midterm	low
Genomics and Proteomics	Genomics data-gathering techniques	medium	midterm	high
	Gene-expression monitoring	medium	midterm	high
	Protein profiling	low	midterm	medium
	Biospectroscopic instruments; terahertz spectroscopy and analysis	low	midterm	high
	Vaccine stratification by genomics and toxicogenomics	high	midterm	high
Therapeutic Drugs and Vaccines	Small-scale vaccine production	high	midterm	low
	Small-molecule and protein therapeutics	low	midterm	high
	Genomics-based vaccine developments	high	midterm	high
	Shock therapeutics	high	midterm	medium
Drug Delivery	Biocapsules	low	midterm	high
	Implantable antidotes	medium	midterm	medium
	Somatic gene therapy	low	far term	high

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warning. It is also possible that development of a particular biotechnology by a potential adversary would increase its importance to the Army.

Recommendation 4. The Army should monitor near-term developments in all of the biotechnology areas listed in Table 8-2, regardless of the investment priority. The list should be updated to accommodate new opportunities as they arise.

BARRIERS NOT AMENABLE TO RESEARCH

The committee identified several barriers to the development of biotechnologies that could not readily be overcome by more research. These include:

- collection mechanisms for target threat molecules
- ethical and privacy issues that could limit the application of genomics and other biotechnologies and public perception that genetically modified organisms are undesirable
- increasing globalization of development and manufacturing expertise
- certification of biomaterials and nonmedical devices
- length of clinical trials required for development of vaccines

Conclusion 5. Miniaturized, biologically based sensing devices could significantly counter "unseen" threats on the battlefield. Timely sensing of biological, as opposed to chemical, agents will require a broad-based network of both internal and external sensing devices. These devices will require development of micro/nanotechnologies, as well as testing facilities to validate the resulting products. Many of the necessary micro/nanotechnologies will only be developed in response to clearly defined Army (or other DOD) requirements.

Recommendation 5. To influence the direction of commercial developments, the Army should immediately devise strategic and tactical concepts for the detection of target threat molecules and identify Army-unique battlefield requirements for internal (health monitoring) and external (environmental monitoring) sensors. The tactical concepts should address sensing, monitoring, and networking capabilities, as well as interfaces with tactical intelligence systems.

Conclusion 6. The Army can take advantage of commercial developments in gene-expression monitoring and protein-profiling systems and techniques that could lead to devices and technologies for monitoring threats to soldiers in the field (as mirrored via gene expression in response to external stimuli) and provide a foundation for new methods of improving soldier training and performance.

Recommendation 6a. The Army should optimize gene-expression-monitoring techniques for soldier applications, especially for the detection of target threat molecules through toxicogenomics.

Recommendation 6b. The Army should develop predictors of individualized immune responses to vaccines so that they can be tailored to genotypes. It should lead the way in laying the groundwork for the open, disciplined use of genomic data to enhance soldiers' health and to improve their performance on the battlefield.

Conclusion 7. The Army, and the country as a whole, are becoming increasingly dependent on foreign sources for many critical therapeutic materials, such as wound treatments, vaccines, and pharmaceuticals. At the same time, federal and state regulations have restricted both military and civilian research and development in therapeutics. In exceptional circumstances, national defense needs might warrant special dispensation from these regulations, and the Army should have legal recourse for requesting exceptions. For example, in urgent cases, the Army simply cannot wait until developers can meet the extremely high (>99.99 percent) effectiveness demanded by federal regulators and civilian consumers for new therapeutics. In such cases, the development of vaccines and antidotes, quasimedical devices, and biotechnology products for nonmedical uses could be accelerated to meet specific military requirements.

Recommendation 7a. Although the cost of investing in manufacturing infrastructure would be prohibitive, the Army should develop and maintain a database of global manufacturing capabilities, including the biology, processes, and equipment necessary to produce critical therapeutic materials. This database should also include key upstream and downstream aspects of the pharmaceutical industry, such as the status of clinical trials.

Recommendation 7b. The Army should define and petition the government to certify special processes for rapid development and approval of biotechnology applications that meet exceptional Army and other defense needs. The Army and the Department of Defense must have the ability to identify exceptional requirements and expedite the development of products that could potentially benefit soldiers confronted with an urgent threat or special need.

Conclusion 8. Developments in cell biology, immunology, molecular genetics, and genomics have led to new concepts that could greatly improve the safety and efficacy of vaccines and reduce the time and lower the cost of vaccine de-

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velopment and production. The Army must be able to respond to threats with vaccines and antibiotics in weeks rather than years. As the pace of genomics advances quickens, the Army will be hard pressed to take advantage of the many opportunities for providing better vaccines more quickly. Reducing the time involved in clinical trials, which routinely involve large populations, should be a high priority.

Recommendation 8a. The Army should build on its strengths in the development of vaccines by funding new technological approaches that could shorten the time for the development and production of vaccines in response to observed pathogens. These include engineered virus-based vaccines and other genomics developments, such as DNA vaccines, cell-based vaccines, and monoclonal antibodies.

Recommendation 8b. The Army should explore (1) using transgenics to shorten the clinical trial phase for defining toxicity and (2) using pharmacogenomics to shorten the time for Phase III clinical trials.

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References

Adleman, L. 1994. Molecular computation of solutions to combinatorial problems. Science 266(5187): 1021–1024.

Agnew, W., and D.B. McCreery, eds. 1990. Neural Prostheses: Fundamental Studies. New York: Prentice-Hall.

Aksay, I.A., and S. Weiner. 1998. Biomaterials: is this really a field of research? Solid State and Materials Science 3(3): 219–220.

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson. 1989. The Molecular Biology of the Cell, 2nd edition. New York: Garland Publishing.

Anderson, L., and J. Seilhamer. 1997. A comparison of selected mRNA and protein abundances in human liver. Journal of Electrophoresis 18: 533–537.

Appenzeller, T. 1990. Democratizing the DNA sequence. Science 247(4946): 1030–1032.

Arnheim, N., and C.H. Levenson. 1990. Polymerase chain reaction. Chemical and Engineering News 68(40): 38–47.

Averner, M., M. Karel, and R. Radner. 1984. Problems associated with utilization of algae in bioregenerative life support systems. NASA Contractor Report 166615. Washington, D.C.: National Aeronautics and Space Administration.

Badylak, S.F. 2000. Personal communications between Stephen F. Badylak, Senior Research Scientist, Department of Biomedical Engineering, Purdue University, and Michael Ladisch, Chair, Committee on Opportunities in Biotechnology for Future Army Applications, June, 2000.

Baer, E., A. Hiltner, and H.D. Keith. 1987. Hierarchical structure in polymeric materials. Science 235(4792): 1015–1022.

Bai, Q., and K. Wise. 2000. A high-yield microassembly structure for three-dimensional microelectrode arrays. IEEE Transactions in Biomedical Engineering 47(3): 281–289.

Ballantine, D.S., R.M. White, S.J. Martin, E.T. Zellers, and H. Wohltijen. 1997. Acoustic Wave Sensors: Theory, Design, and Physico-Chemical Applications. New York: Academic Press.

Barth, P.W., C.C. Beatty, L.A. Field, J.W. Baker, and G.B. Gordon. 1994. A robust normally-closed silicon microvalve. Pp. 248–251 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Bashir, R., M.R. Ladisch, R. Gomez, A. Sarikaya, J. Sturgis, and J.P Robinson. (in press). Adsorption of Avidin on micro-fabricated surfaces for protein biochip applications. Biotechnology and Bioengineering.

Bhatia, S. 1999. Microfabrication in Tissue Engineering and Bioartificial Organs. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Binette, F., D.P. McQuaid, D.R. Haudenschild, P.C. Yaeger, J.M. McPherson, and R. Tubo. 1998. Expression of a stable articular cartilage phenotype without evidence of hypertrophy by adult human articular chondrocytes in vitro. Journal of Orthopedic Research 16(2): 207–216.

BIO (Biotechnology Industry Organization). 2000a. A timeline of biotechnology. Available on line: *http://www.bio.org/timeline/timeline.html* [September 22, 2000].

BIO. 2000b. Industry statistics. Available on line: *http://www.bio.org/aboutbio/guide2000/statistics.html* [September 22, 2000].

Birge, R.R. 1992. Protein based optical computing and optical memories. Computer 25(11): 56–67.

Birge, R.R., P.A. Fleitz, R.B. Gross, J.C. Izgi, A.F. Lawrence, J.A. Stuart, and J.R. Tallent. 1990. Spatial light modulators and optical associative memories based on bacteriorhodopsin. Pp. 1788–1789 in Proceedings of the Twelfth Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Piscataway, N.J.: Institute of Electrical and Electronics Engineers.

Birge, R.R., B. Parsons, Q.W. Song, and J.R. Tallent. 1997. Protein-based three-dimensional memories and associative processors. Pp. 439–471 in Molecular Electronics, edited by M.A. Ratner and J. Jortner. Oxford, U.K.: Blackwell Science, Ltd.

Birge, R.R., N.B. Gillespie, E.W. Izaguirre, A. Kusnetzow, A.F. Lawrence, D. Singh, Q.W. Song, E. Schmidt, J.A. Stuart, S. Seetharaman, and K.J. Wise. 1999. Biomolecular electronics: protein-based associative processors and volumetric memories. Journal of Physical Chemistry 103B(49): 10746–10766.

Black, J. 1999. Biological Performance of Materials, 3rd edition. New York: Marcel Dekker.

Blackstock, W.P., and M.P. Weir. 1999. Proteomics: quantitative and physical mapping of cellular proteins. Trends in Biotechnology 17 (3): 121–127.

Boone, T., and H. Hooper. 1998. Multiplexed, disposable, plastic microfluidic systems for high-throughput applications. Pp. 257–260 in Micro Total Analysis Systems '98, Proceedings of the mTAS '98 Workshop. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Borkholder, D. 1999. Cell-based biosensors using microelectrodes. Stanford, Calif.: Stanford University Press.

Boxer, S.G., J. Stocker, S. Franzen, and J. Salafsky. 1992. Re-engineering photosynthetic reaction centers. Pp. 226–241 in Molecular Electronics Science and Technology, edited by A. Aviram. New York: American Institute of Physics.

Bräuchle, C., N. Hampp, and D. Oesterhelt. 1991. Optical applications of bacteriorhodopsin and its mutated variants. Advanced Materials 3 (9): 420–428.

Brazzle, J.D., I. Papautsky, and A.B. Frazier. 2000. Hollow metallic micromachined needle arrays. Biomedical Microdevices 2(3): 197–205.

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Opportunities in Biotechnology for Future Army Applications
http://www.nap.edu/catalog/10142.html

Brent, R. 2000. Genomic biology. Cell 100(1): 169–183.

Bruno, A., E. Baer, R. Volkel, and C. Effenhauser. 1998. Microoptical fluorescence detection for chip-based multiplexed analysis systems. Pp. 281–285 in Micro Total Analysis Systems '98, Proceedings of the mTAS '98 Workshop. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Buettner, J.A., C.A. Dadd, G.A. Baumbach, B.L. Masecar, and D.J. Hammond. 1996. Chemically derived peptide libraries: a new resin and methodology for lead identification. International Journal of Peptide Protein Research 47(1-2): 70–83.

Bunkin, F.V., A.B. Druzhko, B.I. Mitsner, A.M. Prokhorov, V.V. Savranskii, T.B. Shevchenko, N.W. Tkachenko, and N.N. Vsevolodov. 1981. Diffraction efficiency of bacteriorhodopsin and its analogs. Soviet Technical Physics Letters 7: 630–631.

Burns, M.A., B.N. Johnson, S.N. Brahmasandra, K. Handique, J.R. Webster, M. Krishnan, T.S. Sammarco, P.M. Man, D. Jones, D. Heldsinger, C.H. Mastrangelo, and D.T. Burke. 1998. An integrated nanoliter DNA analysis device. Science 282(5388): 484–487.

Cannon, L.E., R.C. Ladner, and D. McCoy. 1996. Phage-display technology. IVD Technology 2(6): 22.

Carlen, E., and C. Mastrangelo. 1999. A simple, high-actuation-power, thermally-activated paraffin microactuator. Pp. 1364–1367 in Proceedings of Transducers '99. Piscataway, N.J.: Institute of Electrical and Electronics Engineers.

Carter, R., and J. Houk. 1993. Multiple single-unit recordings from the CNS using thin-film electrode arrays. IEEE Transactions in Rehabilitation Engineering 1(3): 175–184.

Chapin, J.K., K.A. Moxon, R.S. Markowitz, and M.A. Nicolelis. 1999. Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. Nature Neuroscience 2(7): 664–670.

Chen, Z., and R.R. Birge. 1993. Protein based artificial retinas. Trends in Biotechnology 11(7): 292–300.

Christel, L.A., K. Peteresen, W.A. McMillan, and M.A. Northrup. 1998. Rapid, automated nucleic acid probe assays using silicon microstructures for nucleic acid concentration. Journal of Biomedical Engineering 121: 22–25.

CID (Center for International Development). 2000. The Case for a Vaccine Purchase Fund. Available on line: *http://www.cid.harvard.edu/malaria/malaria.htm* [March 23, 2001].

Clarke, D.L., C.B. Johansson, J. Wilbertz, B. Veress, E. Nilsson, H. Karlstrom, U. Lendahl, and J. Frisen. 2000. Generalized potential of adult neural stem cells. Science 288(5471): 1660–1663.

Colognato, H., D.E. Winkelmann, and P. Yurchenco. 1999. Laminin polymerization induces a receptor-cytoskeleton network. Journal of Cell Biology 145(3): 619–631.

Corning, Inc. 2000. Corning to enter fast-growing market for DNA microarrays used in genomic research. News release September 12, 2000. Available on line: *http://www.corning.com/cmt/whatsnew/businessrelease.asp* [January 21, 2001].

DA (Department of the Army). 1998. Fiscal Year 1998 Army Science and Technology Master Plan. Assistant Secretary of the Army (Research, Development, and Acquisition). Washington, D.C.: Department of the Army.

de Haard, H.J., N. van Neer, A. Reurs, S. Hufton, R.C. Roovers, P. Henderikx, A.P. de Bruine, J.W. Arends, and H.R. Hoogenboom. 1999. A large non-immunized human fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. Journal of Biological Chemistry 274(26): 18218–18230.

DeLucas, L. 2000. Briefing by L. DeLucas, director of the Center for Macromolecular Crystallography, University of Alabama at Birmingham, to Michael Ladisch, chair, Committee on Opportunities in Biotechnology for Future Army Applications, Lyndon B. Johnson Space Flight Center, Houston, Texas, June 23, 2000.

DeRisi, J.L., R.I. Vishwanath, and P.O. Brown. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278(5338): 680–686.

Desai, T.A., D.J. Hansford, L. Kulinsky, A.H. Nashat, G. Rasi, J. Tu, Y. Wang, M. Zhang, and M. Ferrari. 1999. Nanopore technology for biomedical applications. Biomedical Microdevices 2(1): 11–41.

DeWitt, S., and D. Pfost. 1999. Industrialization of drug discovery and diagnostics with microfluidic chips and pharmacogenetics. Journal of the Association of Laboratory Automation 4(2): 54–57.

DOD (U.S. Department of Defense). 1998. Total Force Anthrax Vaccination Decision Announced. Office of the Assistant Secretary of Defense (Public Affairs) News Release 255-98. Available on line: *http://www.defenselink.mil/news/May1998/b05221998_bt255-98.html* [2000, November 20].

DOE (U.S. Department of Energy). 1999. The Technology Roadmap for Plant/Crop-Based Renewable Resources 2020: Research Priorities for Fulfilling a Vision to Enhance U.S. Economic Security Through Renewable Plant/Crop-Based Resource Use. Washington, D.C.: U.S. Department of Energy, Office of Industrial Technologies.

Duffy, D., J. McDonald, O. Schueller, and G. Whitesides. 1998. Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). Analytical Chemistry 70(23): 4974–4984.

Dunbar, B. 2000. Briefing by B. Dunbar, assistant director, NASA Johnson Space Center to Mike Ladisch, chair, Committee on Opportunities in Biotechnology for Future Army Applications, Lyndon B. Johnson Space Center, Houston, Texas, June 23, 2000.

Ehrfeld, W. 2000. Microreaction Technology: Industrial Prospects. Berlin: Springer-Verlag.

Ellington, A.D., and J.W. Szostak. 1990. In vitro selection of RNA molecules that bind specific ligands. Nature 346(6287): 818–822.

Erlebacher, A., E.H. Filvaroff, S.E. Gitelman, and R. Derynck. 1995. Toward a molecular understanding of skeletal development. Cell 80(3): 371–378.

Fan, H., P. York, and S. Cherukuri. 1997. Chip fabrication for combinatorial chemistry. Pp. 86–96 in Proceedings of the 3rd International Symposium on Microstructures and Microfabricated Systems. Pennington, N.J.: Electrochemical Society.

Ferrari, M., V.T. Granik, A. Imam, and J. Nadeau. 1997. Advances in Doublet Mechanics. New York: Springer-Verlag.

Forbes, N. 2000. Personal communications between Nancy Forbes, senior member of the Technical Staff, Litton TASC and Robert Birge, member of the Committee on Opportunities in Biotechnology for Future Army Applications, July 2000.

Gentzkow, G.D., S.D. Iwasaki, K.S. Hershon, M. Mengel, J.J. Prendergrast, J.J. Ricotta, D.P. Steed, and S. Lipkin. 1996. Use of dermagraft, a cultured human dermis, to treat diabetic foot ulcers. Diabetes Care 19(4): 350–354.

Gergely, C., C. Ganea, G. Groma, and G. Varo. 1993. Study of the photocycle and charge motions of the bacteriorhodopsin mutant D96N. Biophysical Journal 65(6): 2478–2483.

Geyer, C.R., and R. Brent. 2000. Selection of genetic agents from random peptide aptamer expression libraries. Methods in Enzymology 328: 171–208.

Gifford, D. 1994. On the path to computation with DNA. Science 266(5187): 993–994.

Gimzewski, J.K., C. Gerber, E. Meyer, and R.R. Schlittler. 1994. Observation of a chemical reaction using a micromechanical sensor. Chemical Physics Letter 217(5-6): 589–594.

Gomes, Y.M. 1997. PCR and sero-diagnosis of chronic Chagas' disease. Applications in Biochemistry and Biotechnology 66(2): 107–119.

Gourley, S.R. 2000. Future combat systems: a revolutionary approach to combat victory. Army 50(7): 23–26.

Greenbaum, E. 2000. Personal communications between Elias Greenbaum, Oak Ridge National Laboratory, and Robert Birge, member of the Committee on Opportunities in Biotechnology for Future Army Applications, June 2000.

Gulati, M., M.R. Ladisch, R. Hespell, and R.J. Bothast. 1996. Assessment of ethanol production options for corn products. Bioresource Technology 58(3): 253–264.

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Opportunities in Biotechnology for Future Army Applications
http://www.nap.edu/catalog/10142.html

Guo, J., G. Jorudin, and D.K. MacCallum. 1989. Culture and growth characteristics of chondrocytes encapsulated in alginate beads. Connective Tissue Research 19(2-4): 277–297.

Halverson, D.C., A.J. Pyzik, and I.A. Aksay. 1989. Processing of boron carbide-aluminum composites. Journal of the American Ceramic Society 72(5): 775–780.

Hampp, N., A. Popp, C. Bräuchle, and D. Oesterhelt. 1992. Diffraction efficiency of bacteriorhodopsin films for holography containing bacteriorhodopsin wildtype BRwt and its variants BR_{D85E} and BR_{D96N}. Journal of Physical Chemistry 96(11): 4679–4685.

Hampp, N., R. Thoma, D. Zeisel, and C. Bräuchle. 1994. Bacteriorhodopsin variants for holographic pattern recognition. Advances in Chemistry 240: 511–526.

Handique, K., B. Gogoi, D. Burke, C. Mastrangelo, and M. Burns. 1997. Microfluidic flow control using selective hydrophobic patterning. Proceedings of SPIE 3224: 185–199.

Harsanyi, G. 1995. Polymer Films in Sensor Applications. Lancaster, U.K.: Technomic Publishing.

Hendrickson, W.A. 1986. X-ray diffraction methods for the analysis of metalloproteins. Pp. 215–259 in Physical Methods for Inorganic Chemistry, edited by J.R. Wright, W.A. Hendrickson, S. Osaki, and G.T. James. New York: Plenum Publishing Corporation.

Hendrickson, W.A. 1987. X-ray diffraction. Pp. 5–13 in Protein Engineering, edited by D.L. Oxender and C.F. Fox. New York: Alan R. Liss, Inc.

Hoke, C. 2000. Army Infectious Disease Research. Briefing by COL C. Hoke, Research Area Director, U.A. Army Medical Research and Materiel Command, to the Committee on Opportunities in Biotechnology for Future Army Applications, Sheraton National Hotel, Arlington, Virginia, February 17, 2000.

Horbett, T.A., and J.L. Brash. 1995. Proteins at Interfaces II. New York: Oxford University Press.

Howe, R.T., and R.S. Muller. 1986. Resonant-microbridge vapor sensor. IEEE Transactions on Electronic Devices 33: 499–506.

Hughes, R.C., M.P. Eastman, W.G. Yelton, A.J. Ricco, S.V. Patel, and M.W. Jenkins. 1998. Application of the solubility parameter concept to the design of chemiresistor arrays. Pp. 379–382 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Ingber, D. 1997a. Tensegrity: the architectural basis of cellular mechanotransduction. Annual Review of Physiology 59: 575–599.

Ingber, D. 1997b. Extracellular Matrix: A Solid-State Regulator of Cell Form, Function, and Tissue Development. Pp. 541–566 in Handbook of Cell Physiology, edited by J.D. Jamieson and J.F. Hoffman. New York: Oxford University Press.

Inglesby, T.V., D.A. Henderson, J.G. Bartlett, M.S. Ascher, E. Eitzen, A.M. Friedlander, J. Hauer, J. McDade, M.T. Osterholm, T. O'Toole, G. Parker, T.M. Perl, P.K. Russell, and K. Tonat. 1999. Anthrax as a biological weapon: medical and public health management. Journal of the American Medical Association 281(18): 1735–1745.

Jerman, H. 1991. Electrically-activated normally-closed diaphragm valves. Pp. 1045–1048 in Digest IEEE International Conference on Solid-State Sensors and Actuators. Piscataway, N.J.: Institute of Electrical and Electronics Engineers.

Jette, B. 2000. Land Warrior and Soldier Systems. Briefing by COL Bruce Jette, U.S. Program Manager for Soldier Systems, to the Committee on Opportunities in Biotechnology for Future Army Applications, National Research Council, Washington, D.C., April 27, 2000.

Ji, J., and K. Wise. 1992. An implantable CMOS circuit interface for multiplexed microelectrode recording arrays. IEEE Journal of Solid-State Circuits 27(3): 433–443.

Keim, C., and M.R. Ladisch. 2000. New system for preparative electrochromatography of proteins. Biotechnology and Bioengineering 70(1): 72–81.

Kennedy, P., and R. Bakay. 1997. Activity of single action potentials in monkey motor cortex during long-term task learning. Brain Research 760(1-2): 251–254.

Kern, P. 2000. Biotechnology: Way to the Future. Briefing by LTG P. Kern, Military Deputy to the Assistant Secretary of the Army (Acquisition, Logistics, and Technology), to the Committee on Opportunities in Biotechnology for Future Army Applications, National Research Council, Washington, D.C., April 27, 2000.

Kim, G.H., M. Sarikaya, D.L. Milius, and I.A. Aksay. 1989. Microstructural and fractographic characterization of B_4C-Al cermets tested under dynamic and static loading. Pp. 562–563 in Proceedings of the 4th Annual Meeting EMSA. San Francisco, Calif.: San Francisco Press.

Kohlmann, K.L., P.J. Westgate, A. Velayudhan, J. Weil, A. Sarikaya, M.A. Brewer, R.L. Hendrickson, and M.R. Ladisch. 1996. Enzyme conversion of lignocellulosic plant materials for resource recovery in a controlled ecological life support system. Advances in Space Research 18(1-2): 251–265.

Kong, L.C., B.G. Orr, and K.D. Wise. 1993. Integrated electrostatically-resonant scan tip for an atomic force microscope. Journal of Vacuum Science and Technology 11B(3): 634–641.

Kramer, P. 1993. Listening to Prozac. New York: Viking.

Kusnadi, A.R., E.E. Hood, D.R. Witcher, J.A. Howard, and Z.L. Nikolov. 1998. Production and purification of two recombinant proteins from transgenic corn. Biotechnology Progress 14(1): 149–155.

Ladisch, M.R. 2001. Bioseparations Engineering: Principles, Practice and Engineering. New York: John Wiley and Sons.

Ladisch, M.R. In press. Bioprocess Engineering, in Van Nostrand Scientific Encyclopedia, 9th Edition, edited by P.H. Kulik. New York: John Wiley and Sons.

Laitin, E.A., and E.M. Pelletier. 1997. The Influenza A/New Jersey (Swine Flu) Vaccine and Guillain-Barré Syndrome: The Arguments for a Causal Association. Available on line: *http://www.hsph.harvard.edu/Organizations/ddil/swineflu.html* [December 4, 2000].

Lakes, R. 1993. Materials with structural hierarchy. Nature 361(6412): 511–515.

Lang, H.P., M.K. Baller, F.M. Battiston, J. Fritz, R. Berger, J.P. Ramseyer, P. Fornaro, E. Meyer, H.J. Guntherodt, J. Brugger, U. Drechsler, H. Rothuizen, M. Despont, P. Vettiger, Ch. Gerber, and J.K. Gimzewski. 1999. The nanomechanical NOSE. Pp. 9–13 in Digest IEEE International Conference on MicroElectroMechanical Systems. Piscataway, N.J: Institute of Electrical and Electronics Engineers.

Langmuir, A.D. 1979. Guillain-Barré syndrome: the swine influenza virus vaccine incident in the United States of America, 1976–1977: preliminary communication. Journal of the Royal Society of Medicine 72(19): 660–669.

Langridge, W.H.R. 2000. Edible vaccines. Scientific American 283(3): 66–71.

Lee, I., J.W. Lee, and E. Greenbaum. 1997. Biomolecular electronics: vectorial arrays of photosynthetic reaction centers. Physical Review Letters 79: 3294–3297.

Lin, Y., N. Xu, J. Wen, D. Matson, and R.D. Smith. 1998. Microfabricated dual-microdialysis and capillary isoelectric focusing devices for cleanup and separations/mass spectrometric analysis of biomolecules. Pp. 343–346 in Micro Total Analysis Systems '98, Proceedings of the mTAS '98 Workshop. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Linton, R.H. 2001. Personal communication between Richard H. Linton, Associate Professor in the Department of Food Sciences, Purdue University and Michael Ladisch, Chair, Committee on Opportunities in Biotechnology for Future Army Applications, January, 2001.

Lowenstam, H., and S. Weiner. 1989. On Biomineralization. New York and Oxford, U.K.: Oxford University Press.

Majoo, S., J.W. Schwank, J.L. Gland, and K.D. Wise. 1995. A selected-area CVD method for deposition of sensing films on monolithic integrated gas detectors. IEEE Electron Device Letters 16(6): 217–219.

Man, P., C. Mastrangelo, M. Burns, and D. Burke. 1998. Microfabricated capillary-driven stop valve and sample injector. Pp. 45–50 in Proceed-

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Opportunities in Biotechnology for Future Army Applications
http://www.nap.edu/catalog/10142.html

ings of the IEEE Micro Electro Mechanical Systems Workshop. Piscataway, N.J.: Institute of Electrical and Electronics Engineers.

Mastrangelo, C.H., M.A. Burns, and D.T. Burke. 1998. Microfabricated devices for genetic diagnostics. Proceedings of the IEEE 86(8): 1769.

Matsumoto, K., M. Ishii, K. Segawa, Y. Oka, B.J. Vartanian, and J.S. Harris. 1996. Room temperature operation of a single electron transistor made by the scanning tunneling microscope nanooxidation process for the TiOx/Ti system. Applied Physics Letters 68 (1): 34–36.

McBride, S., R. Moroney, and W. Chiang. 1998. Electrohydrodynamic pumps for high-density microfluidic arrays. Pp. 45–48 in Micro Total Analysis Systems '98, Proceedings of the mTAS '98 Workshop. Dordrecht, The Netherlands: Kluwer Academic Publishers.

McLaren, A. 2000. Cloning: pathways to a pluripotent future. Science 288(5472): 1775–1780.

Miercke, L.J.W., M.C. Betlach, A.K. Mitra, R.F. Shand, S.K. Fong, and R.M. Stroud. 1991. Wild-type and mutant bacteriorhodopsins D85N, D96N, and R82Q: purification to homogeneity, pH dependence of pumping and electron diffraction. Biochemistry 30(12): 3088–3098.

Misra, S., R. Govindjee, T.G. Ebrey, N. Chen, J.-X. Ma, and R.K. Crouch. 1997. Proton uptake and release are rate-limiting steps in the photocycle of the bacteriorhodopsin mutant E204Q. Biochemistry 36(16): 4875–4883.

Mitchell, L.S., S. Nielsen, P. Nelson, P. Trumbo, T. Hodges, P. Hasegawa, R. Bressan, M. Ladisch, and D. Auslander. 1995. Earth benefits of interdisciplinary CELSS-related research by the NSCORT in bioregenerative life support. Advances in Space Research 18(4-5): 23–31.

Miyasaka, T., K. Koyama, and I. Itoh. 1992. Quantum conversion and image detection by a bacteriorhodopsin-based artificial photoreceptor. Science 255(5298): 342–344.

Moore, J.M. 2000. Personal communications between Julie M. Moore, assistant professor, Center for Tropical and Emerging Global Diseases and Department of Medical Microbiology and Parasitology, College of Veterinary Medicine, University of Georgia, and Janet Westpheling, member of the Committee on Opportunities in Biotechnology for Future Army Applications, June 2000.

Mourlas, D., A. Jaeggi, B. Flannery, B. Gray, C. van Drieenhuizen, N. Storment, N. Maluf, and G. Kovacs. 1998. Novel interconnection and channel technologies for microfluidics. Pp. 27–30 in Micro Total Analysis Systems '98, Proceedings of the mTAS '98 Workshop. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Mrksich, M., and G. Whitesides. 1996. Using self-assembled monolayers to understand the interactions of man-made surfaces with proteins and cells. Annual Review of Biophysics and Biomolecular Structures 25: 55–78.

Mueller, C.D., S. Nazarenko, and T. Ebeling. 1997. Novel structures by microlayer coextrusion-talc-filled PP, PC/SAN, and HDPE/LLDPE. Polymer Engineering Science 37(2): 355–362.

Mundlos, S., and B.R. Olsen. 1997. Heritable diseases of the skeleton. Part I. molecular insights into skeletal development-transcription factors and signaling pathways. The Federation of American Societies for Experimental Biology Journal 11(2): 125–132.

Najafi, K., K. Wise, and T. Mochizuki. 1985. A high-yield IC compatible process for fabrication of microprobes. IEEE Transactions on Electron Devices 35: 1206–1211.

Najafi, N., K.D. Wise, and J.W. Schwank. 1994. A micromachined ultra-thin-film gas detector. IEEE Transactions on Electron Devices 41(10): 1770–1777.

Nashat, A.H., M. Moronne, and M. Ferrari. 1998. Detection of functional groups and antibodies on micro-fabricated surfaces by confocal microscopy. Biotechnology Bioengineering 60(2): 137–146.

Nichol, S. T., C. F. Spiropoulo, S. Morzunou, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, and C. F. Peters. 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262(5135): 914–918.

NIH BECON (National Institutes of Health Bioengineering Consortium). 2000. Nanoscience and Nanotechnology: Shaping Biomedical Research, Symposium Report, June, 2000. Bethesda, Md.:, National Institutes of Health.

NSTC (National Science and Technology Council). 2001. National Nanotechnology Initiative: Leading to the Next Industrial Revolution. Committee on Technology, Interagency Working Group on Nanoscience, Engineering and Technology. Washington, D.C.: National Science and Technology Council.

Oesterhelt, D., C. Bräuchle, and N. Hampp. 1991. Bacteriorhodopsin: a biological material for information processing. Quarterly Reviews of Biophysics 24(4): 425–478.

Olson, S. 1986. Biotechnology: An Industry Comes of Age. Washington, D.C.: National Academy Press.

Osbourn, G.C., J.W. Bartholomew, G.C. Frye, and A.J. Ricco. 1994. Clustering-based pattern recognition applied to chemical recognition using SAW array signals. Pp. 193–196 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Pääbo, S., Higuchi, R.G. and A.C. Wilson. 1989. Ancient DNA and the polymerase chain reaction. Journal of Biological Chemistry 264(17): 9709–9712.

Paek, E.G., and D. Psaltis. 1987. Optical associative memory using Fourier transform holograms. Optical Engineering 26: 428–433.

Pennisi, E., and G. Vogel. 2000. Clones: a hard act to follow. Science 288 (5472): 1722–1726.

Petersen, K.E. 2000. Bringing MEMS to Market. Pp. 60–64 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Petsko, G.A. 2000. Personal communications between Gregory A. Petsko, Gyula and Katica Tauber Professor of Biochemistry and Chemistry and Director, Rosenstiel Basic Medical Sciences Research Center, Brandeis University and Janet Westpheling, member of the Committee on Opportunities in Biotechnology for Future Army Applications, July, 2000.

Pourahmadi, F., M. Taylor, G.A. Kovacs, K. Lloyd, S. Sakai, T. Schafer, B. Helton, L. Western, S. Zaner, J. Ching, W.A. McMillan, P. Belgrader, and M.A. Northrup. 2000. Toward a rapid, integrated, and fully automated DNA diagnostic assay for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Clinical Chemistry 46(9): 1511–1513.

Quate, C.F. 2000. Cantilever Nanotechnologies. Briefing by C.F. Quate, Leland T. Edwards Professor in the School of Engineering and Professor (Research) of Applied Physics, Stanford University, to the Committee on Opportunities in Biotechnology for Future Army Applications, Hyatt Rickeys Hotel, Palo Alto, California, March 30, 2000.

Rawls, R.L. 1999. Taking a clue from biology. Chemical and Engineering News 77(15): 38–43.

Rhem, K.T. 2000. Tomorrow's grunts need to be cream of crop. Armed Forces Press Service news release. Available on line: *http://www.defenselink.mil/news/Aug2000/n08312000_20008314.html* [December 7, 2000].

Ricco, A.J., R.M. Crooks, and G.C. Osbourn. 1998. Surface acoustic wave chemical sensor arrays: new chemically sensitive interfaces combined with novel cluster analysis to detect volatile organic compounds and mixtures. Accounts of Chemical Research 31(5): 289–296.

Rich, C., and K. Wise. 2000. A thermopneumatically-actuated microvalve with improved thermal efficiency. Pp. 234–237 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Roberts, R.W., and J.W. Szostak. 1997. RNA-peptide fusions for the *in vitro* selection of peptides and proteins. Proceedings of the National Academy of Sciences USA 94: 12297–12302.

Rosamond, J., and A. Allsop. 2000. Harnessing the power of the genome in the search for new antibiotics. Science 287(5460): 1973–1976.

Rousche, P.J., and R.A. Normann. 1998. Chronic recording capability of the Utah intracortical electrode array in cat sensory cortex. Journal of Neuroscience and Methods 82(1): 1–15.

Rudolph, A.S. 2000. Harvesting Biology for Future Defense. Briefing by Dr. Alan Rudolph, program manager for the Controlled Biological and

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Opportunities in Biotechnology for Future Army Applications
http://www.nap.edu/catalog/10142.html

Biomimetic Systems, Tissue-Based Biosensors, Activity Detection Technologies and Advanced Diagnostics Programs, Defense Advanced Research Projects Agency, to the Committee on Opportunities in Biotechnology for Future Army Applications, Sheraton National Hotel, Arlington, Virginia, February 16, 2000.

Santini, J.T., M.J. Cima, and R. Langer. 1999. A controlled-release microchip. Nature 397(6717): 335–338.

Sarikaya, M., and I.A. Aksay. 1992. Nacre of Abalone Shell: A Natural Multifunctional Nanolaminated Ceramic-Polymer Composite Material. Pp. 1–26 in Structure, Cellular Synthesis and Assembly of Biopolymers, edited by S.T. Case. New York: Springer-Verlag.

Sarikaya, M., and I.A. Aksay. 1995. Biomimetics: Design and Processing of Materials. Woodbury, N.Y.: AIP Press.

Schwarzbauer, J. 1999. Basement membranes: putting up the barriers. Current Biology 9(7): R242–R244.

Shen, Y., C.R. Safinya, K.S. Liang, A.F. Ruppert, and K.J. Rothschild. 1993. Stabilization of the membrane protein bacteriorhodopsin to 140°C in two-dimensional films. Nature 366(6450): 48–50.

Siegel, R.W. 1993. Exploring mesoscopia: the bold new world of nanostructures. Physics Today 46: 64–68.

Sindrup, S.H., and K. Brosen. 1995. The pharmacogenetics of codeine hypoalgesia. Pharmacogenetics 5(6): 335–346.

Smith, B.L., T.E. Schaffer, M. Viani, J.B. Thompson, N.A. Frederick, J. Kindt, A. Belcher, G.D. Stucky, D.E. Morse, and P.A. Hansma. 1999. Molecular mechanistic origin of the toughness of natural adhesives, fibers and composites. Nature 399(6738): 761–763.

Smith G.D., E. Ciszak, and W. Pangborn. 1996. A novel complex of a phenolic derivative with insulin: structural features related to the T—>R transition. Protein Science 5(8):1502–1511.

Snow, E.S., and P.M. Campbell. 1995. AFM fabrication of sub-10-nanometer metal-oxide devices with in situ control of electrical properties. Science 270(5242): 1639–1641.

Stuart, J.A. 2000. Personal communications between Jeffrey A. Stuart, research assistant professor of biophysics, Syracuse University, and Robert Birge, member of the Committee on Opportunities in Biotechnology for Future Army Applications, November 2000.

Studt, T. 1999. Development of microfluidic UHTS systems speeding up. R&D Magazine 41(2): 43.

Tan, E.H.L., D.S.K. Govender, and R.R. Birge. 1996. Large organic cations can replace Mg^{2+} and Ca^{2+} ions in bacteriorhodopsin and maintain proton pumping ability. Journal of the American Chemical Society 118: 2752–2753.

Terret, N.K., M. Gardner, D.W. Gordon, R.J. Kobylecki, and J. Steele. 1995. Combinatorial synthesis: the design of compound libraries and their application to drug discovery. Tetrahedron 51(30): 8135–8173.

Terry, S.C., J.H. Jerman, and J.B. Angell. 1979. A gas chromatographic air analyzer fabricated on a silicon wafer. IEEE Transactions in Electron Devices 26: 1880–1886.

USAF (U.S. Air Force). 1998. DOD Decides on Total Force Anthrax Vaccination. Air Force News Service news release. Available on line: *http://www.af.mil/news/May1998/n19980522_980715.html* [November 20, 2000].

Velayudhan, A., K.L. Kohlmann, P.J. Westgate, and M.R. Ladisch. 1995. Analysis of plant harvest indices for bioregenerative life support systems. Enzyme and Microbial Technology 17(10): 907–910.

Von Arx, J., and K. Najafi. 1999. A single-chip fully-integrated telemetry-powered system for peripheral nerve stimulation. Pp. 215–215 Proceedings of the IEEE International Solid-State Circuits Conference (ISSCC '99). Piscataway, N.J.: Institute of Electrical and Electronics Engineers.

Vsevolodov, N.N. 1998. Biomolecular Electronics: An Introduction via Photosensitive Proteins. Boston: Birkhauser.

Vsevolodov, N.N., and V.A. Poltoratskii. 1985. Holograms in biochrome, a biological photochromic material. Soviet Physics Technical Letters 30: 1235.

Wallace, R. 1998. A little too much? Expense versus return in HTS miniaturization. Drug Discovery Today 3(7): 299.

Watkins, B.A. 2001. Personal communications between Bruce A. Watkins, professor and director of the Center for Enhancing Foods to Protect Public Health, Purdue University, and Michael Ladisch, chair, Committee on Opportunities in Biotechnology for Future Army Applications, January 2001.

Watson, J.D., M. Gilman, J. Witkowski, and M. Zoller. 1992. Recombinant DNA, 2nd edition. New York: W.H. Freeman and Company.

Westgate, P.J., K. Kohlmann, R.L. Hendrickson, and M.R. Ladisch. 1992. Bioprocessing in space. Enzyme and Microbial Technology 14(1): 76–79.

WHO (World Health Organization). 1999. The World Health Report: 1999: Making a Difference. Geneva: World Health Organization.

Wise, K.D. 1996. Microelectromechanical systems: interfacing electronics to a non-electronic world. Pp. 11–18 in Technical Digest, Proceedings of the IEEE International Electron Devices Meeting. Piscataway, N.J: Institute of Electrical and Electronics Engineers.

Wolpaw J.R., N. Birbaumer, W.J. Heetderks, D.J. McFarland, P.H. Peckham, G. Schalk, E. Donchin, L.A. Quatrano, C.J. Robinson, and T.M. Vaughan. 2000. Brain-computer interface technology: a review of the first international meeting. IEEE Transactions in Rehabilitation Engineering 8(2): 164–173.

Wong, S.S., E. Joselevich, A.T. Woolley, C.L. Cheung, and C.M. Lieber. 1998. Covalently functionalized nanotubes as nanometre-sized probes in chemistry and biology. Nature 394(6688): 52–55.

Woolley, A.T., D. Hadley, P. Landre, A.J. deMello, R.A. Mathies, and M.A. Northrup. 1996. Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. Analytical Chemistry 68(23): 4081–4086.

Zdeblick, M.J., R. Anderson, H. Jankowski, B. Kline-Schroeder, L. Christel, R. Miles, and W. Weber. 1994. Thermopneumatically-actuated microvalves and integrated electro-fluidic circuits. Pp. 251–254 in Solid-State Sensor and Actuator Workshop. Cleveland Heights, Ohio: Transducers Research Foundation, Inc.

Zeisel, D., and N. Hampp. 1992. Spectral relationship of light-induced refractive index and absorption changes in bacteriorhodopsin films containing wildtype BR_{wt} and the variant BRD_{96N}. Journal of Physical Chemistry 96(19): 7788–7792.

Zhang, M., T. Desai, and M. Ferrari. 1998. Proteins and cells on PEG-immobilized silicon surfaces. Biomaterials 19(10): 953–960.

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Appendixes

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Appendix A

Biographical Sketches of Committee Members

Michael R. Ladisch, chair, NAE, is the Director of the Laboratory of Renewable Resources Engineering and Distinguished Professor of Agricultural and Biological Engineering and Biomedical Engineering at Purdue University. He earned his B.S. from Drexel University and his M.S. and Ph.D. from Purdue University, all in chemical engineering. Dr. Ladisch's research addresses fundamental topics in bioseparations (e.g., chromatography, electrochromatography), bionanotechnology (e.g., protein biochips, proteins at surfaces, biomimetics), and bioprocessing of renewable resources for value-added products. He has a broad background in bioscience and bioengineering. He previously chaired the National Research Council (NRC) Committee on Bioprocess Engineering.

Ilhan Aksay is professor in the Department of Chemical Engineering and the Princeton Materials Institute at Princeton University and a former director of the Advanced Materials Technology Center at the University of Washington. He is an expert on ceramic-matrix composites and has written extensively on the utilization of biomimetic techniques in materials processing. Dr. Aksay served on the NRC Committee on Synthetic Hierarchical Structures and the NRC Committee on Army Basic Research. He earned his B.S. in ceramic engineering with honors from the University of Washington and his M.S. and Ph.D. in materials science and engineering from the University of California at Berkeley.

Eric Baer is the Herbert Henry Dow Professor in the Department of Macromolecular Science and director of the Center on Hierarchical Structures at Case Western Reserve University. He is a past director of the National Aeronautics and Space Administration Center for the Commercial Development of Space and Materials for Space Structures, and he has written extensively on molecularity, plastics, and polymer materials. Dr. Baer received an M.A. and D. Eng. in chemistry from Johns Hopkins University and he was a member of the NRC Space Applications Board.

Robert R. Birge is Distinguished Professor of Chemistry and the Director of the W.M. Keck Center for Molecular Electronics at Syracuse University. He is also research director for the New York State Center for Advanced Technology in Computer Applications and Software Engineering and research professor in the Department of Ophthalmology at the State University of New York Health Sciences Center. Dr. Birge has a B.S. in chemistry from Yale University and a Ph.D. in chemistry from Wesleyan University. He has published articles on bio-optical materials, holographics, and protein-based computers and was one of the *Time Magazine* Digital Top 50 Cyber Elite in 1997.

Roger Brent is the Director of the Molecular Sciences Institute in Berkeley, California, and an adjunct professor of biopharmaceutical sciences at the University of California, San Francisco. He is a member of the Scientific Advisory Board of Genetics Institute/Wyeth Ayerst Research, chair of the scientific advisory boards for several smaller companies, and advisor to the Program in Bioinformatics at the University of California, Santa Cruz. He also advises a number of U.S. government agencies and is a member of the Functional Genomics Steering Committee for the Wellcome Trust in the United Kingdom. He has published more than 70 articles and has received 12 patents for genetics applications. Dr. Brent received his B.A. in computer science and mathematics from the University of Southern Mississippi and his Ph.D. in biochemistry and molecular biology from Harvard University, where he also completed postdoctoral work and served on the faculty of the Department of Genetics.

Sheila H. Dewitt is the Director for Business Development at ArQule, Inc., Woburn, Massachusetts. She earned her B.A. in chemistry from Cornell University and her Ph.D. in synthetic organic chemistry from Duke University. She has held technical and business development positions at several pharmaceutical, biotechnology, and agricultural chemical companies including FMC Agricultural Chemical Group,

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Parke-Davis Pharmaceutical Research, Diversomer Technologies, and Orchid BioSciences. Dr. DeWitt is a pioneer in combinatorial chemistry and automated synthesis and the recipient of the Michigan Leading Edge Technologies Award, the Pioneer in Laboratory Robotics Award, and the Association for Laboratory Automation Outstanding Service Award. She is the author of more than 35 publications and holds more than 30 patents.

Mauro Ferrari is professor of mechanical engineering, professor of internal medicine (oncology), and the director of the Biomedical Engineering Center at Ohio State University. He is former director of the Biomedical Microdevices Center at the University of California, Berkeley, and an expert in microfabrication of structures and devices, including biosensors. Dr. Ferrari has a Dottore in Matematica from the Universitá di Padova, Italy, and an M.S. and a Ph.D. in mechanical engineering from the University of California, Berkeley. He has conducted extensive research in biomicroelectromechanical systems (bio-MEMS) and nanostructures for biotechnical applications.

Christopher (Kit) C. Green is executive director for research and development and the chief technical officer-Asia Pacific for General Motors Corporation. Dr. Green received his Ph.D. in neurophysiology from the University of Colorado and an M.D. (honors) through the Department of Health, Education, and Welfare, M.D.-Ph.D. Program from Autonomous City University. For more than 15 years he was senior division analyst with the Office of Scientific and Weapons Intelligence of the Central Intelligence Agency. He is the author of more than 50 peer-reviewed technical publications. Dr. Green is former chair of the NRC Committee on Science and Technology for Defense Conversion in the Year 2020 and the former chair of the NRC Board on Army Science and Technology.

Nile F. Hartman is senior vice president and the Chief Technology Officer of Photonic Sensor Systems, Inc. which develops prototype sensor systems for environmental, biomedical, and metrology applications. He is an expert in electro-optic sensing systems and has an extensive background in holography and semiconductor lasers. He is the author or coauthor of more than 50 publications and has been awarded seven patents. He is a fellow of the Optical Society of America. Mr. Hartman received his B.S. in industrial technology, and he studied physics at Ohio State University.

Paul E. Laibinis is an associate professor of chemical engineering at the Massachusetts Institute of Technology (MIT). He was awarded the Presidential Early Career Award for Scientists and Engineers and the Office of Naval Research Young Investigator Award in 1996 and the Camille Dreyfus Teacher-Scholar Award in 1998 for research on the properties and tailorabilities of organic surfaces and thin films. He has published more than 75 professional articles and holds three patents for innovative coatings, surfaces, and microsensors. He is an active member of the American Institute of Chemical Engineers, the American Chemical Society, and the Materials Research Society. Dr. Laibinis received his S.B. in chemistry and S.B. in chemical engineering from MIT and his A.M. and Ph.D. in organic chemistry from Harvard University.

Verne L. (Larry) Lynn is an independent consultant to industry and the Department of Defense. Mr. Lynn retired from the U.S. government as the Director of the Defense Advanced Projects Agency (DARPA), the principal DOD agency for research, development, and demonstration of concepts, devices, and systems for advanced military capabilities. He served as the Deputy Undersecretary of Defense for Advanced Technology and was the Vice President and Chief Operating Officer for the Atlantic Aerospace Electronics Corporation. He is the author of more than 40 technical publications in the areas of military surveillance and weapons systems and has extensive knowledge in military research and development. He is a fellow of the IEEE, a past member of the Army Science Board, and a current member of the Defense Science Board.

M. Allen Northrup is the Vice President and Chief Technical Officer for Cepheid, a developer of biotechnological analysis instrumentation in Sunnyvale, California. Before joining Cepheid, he was principal engineer in the Microtechnology Center at the Lawrence Livermore National Laboratory, where he was involved in research and development of microinstrumentation for analytical biotechnology and actuator-based biomedical devices. Dr. Northrup was an adjunct professor of radiology at the University of California San Francisco Medical School and is currently a consulting professor of electrical engineering in the Center for Integrated Systems at Stanford University. Dr. Northrup received his Ph.D. in biomedical engineering from the University of California at Davis. He has 15 years of instrumentation development experience in both academia and industry.

Thomas C. Ransohoff is the Vice President for Operations at TranXenoGen, Inc., in Shrewsbury, Massachusetts. He has managed operations for scale-up and production of biotechnology products and directed technical and commercial development of a range of separations technologies and products. In addition to process and product development, he has been responsible for technical management and industry and government investments in biotechnology research. He has an S.B. from MIT and an M.S. from the University of California at Berkeley, both in chemical engineering. He has industry experience in the analysis of biopharmaceutical processes, bioseparations, and transgenics.

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Daniel I.C. Wang, NAE, was the director of the Biotechnology Process Engineering Center at the Massachusetts Institute of Technology until 1998. He is currently Institute Professor of Chemical Engineering and professor of biochemical engineering at MIT. His expertise includes biocatalysis, biochemical separation and purification, bioreactors, biosensors, fermentation processes, and biotechnology. Before entering academia, Dr. Wang was a process engineer at the U.S. Army Biological Laboratory and was awarded the U.S. Army Commendation Medal. He has served on several NRC committees including the Committee on Biobased Industrial Products, the Committee on Bioprocess Engineering, and the Committee on Biotechnology. He is a former member of the NRC Board on Biology and the Board on Chemical Science and Technology. Dr. Wang received his B.S. in chemical engineering and his M.S. in biochemical engineering from MIT; he earned his Ph.D. in chemical engineering from the University of Pennsylvania.

Janet Westpheling is an associate professor of genetics at the University of Georgia. Dr. Westpheling received her B.S. in microbiology from Purdue University and her Ph.D. in genetics from the John Innes Institute, and she was a postdoctoral fellow at Harvard University. Her research interests involve the control of gene expression in *Streptomyces*, particularly carbon utilization and primary metabolism and the strategies used by bacteria to regulate genes involved in morphogenesis and secondary metabolism. Dr. Westpheling previously served on the NRC Committee on Biotechnology.

Kensall D. Wise, NAE, is the J. Reid and Polly Anderson Professor of Manufacturing Technology and professor of electrical engineering and computer science at the University of Michigan. He is also the director of the National Science Foundation Engineering Research Center for Wireless Integrated Microsystems and a former member of the technical staff at the Bell Telephone Laboratories. Dr. Wise is an expert in integrated circuit process technology, solid-state sensors, MEMS, microsystems, and integrated electronics. He is a fellow of both the Institute of Electrical and Electronics Engineers and the American Institute of Medical and Biological Engineering. Dr. Wise received his B.S.E.E. (with highest distinction) from Purdue University and both his M.S.E.E. and Ph.D. in electrical engineering, from Stanford University.

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Appendix B

Meetings and Activities

This appendix lists presentations provided to the committee at meetings and fact-finding sessions conducted during the course of this study.

First Committee Meeting, December 14–15, 1999 Washington, D.C.

Statement of Task and Army S&T in Biotechnology
Dr. William E. Morrison
Office of the Deputy Assistant Secretary (Research and Technology)

Army Biotechnology Applications
Dr. James J. Valdes
U.S. Army Soldier and Biological Chemical Command

ONA Recommendations on Military Applications
Dr. Maria Powell
Office of Net Assessment

ARL Research in Biotechnology
Dr. Bruce West
Army Research Office

DARPA Research Activities
Dr. Abraham Lee
Defense Advanced Research Projects Agency

Second Committee Meeting, February 16–17, 2000 Arlington, Virginia

Presentation to the BAST Panel on Biotechnology
COL John F. Glenn
U.S. Army Medical Research and Materiel Command

Military Operational Medicine
LTC Karl E. Friedl
U.S. Army Medical Research and Materiel Command

Medical, Chemical, and Biological Defense
COL Edwin Armitage
U.S. Army Medical Research and Materiel Command

Combat Casualty Care
COL Robert Vandre
U.S. Army Medical Research and Materiel Command

Infectious Disease Research
Dr. Charles Hoke, Jr.
U.S. Army Medical Research and Material Command

Harvesting Biology for Future Defense
Dr. Alan Rudolph
Defense Advanced Research Projects Agency

Third Committee Meeting, April 27–28, 2000 National Academy of Sciences, Washington, D.C.

ONA Seminar on PerformanceEnhancement
Dr. James J. Valdes
U.S. Army Soldier and Biological Chemical Command

Army Biotech and Vision
LTG Paul Kern
Military Deputy to the Assistant Secretary of the Army (Aquisition, Logistics, and Technology)

Soldier Systems for the Land Warrior
COL Bruce Jette
U.S. Army Project Manager for Soldier Systems

Fourth Committee Meeting, June 20–21, 2000 National Research Council, Washington, D.C.

No presentations were made during this meeting.

First Fact-Finding Session, February 7, 2000 Cambridge, Massachusetts

Participants:

Philip Sharp
Massachusetts Institute of Technology

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George Church
Harvard University

George Whitesides
Harvard University

Noubar Afeyan
NewcoGen Group

Irwin Taub
U.S. Army Natick Research, Development and Engineering Center

Gene Herbert
U.S. Army Natick Research, Development and Engineering Center

Steve Arcidiacono
U.S. Army Natick Research, Development and Engineering Center

Frank Lee
Millennium Corporation

Second Fact-Finding Session, March 30, 2000
Hyatt Rickeys Hotel, Palo Alto, California

Participants:

Raymond P. Mariella, Jr.
Lawrence Livermore National Laboratory

Richard Mathies
University of California at Berkeley

Calvin F. Quate
Stanford University

Mike Hunkapiller
Applied Biosystems

Michael Heller
Nanogen

Third Fact-Finding Session, April 13, 2000
Case Western Reserve University, Cleveland, Ohio

Participants:

Stuart Rowan
Case Western Reserve University

Sergei Nazarenko
Case Western Reserve University

Roger Marchant
Case Western Reserve University

Ka Yee Lee
Illinois State University

John Lannut
Case Western Reserve University

Derek Hansford
Ohio State University

James Anderson
Case Western Reserve University

Fourth Fact-Finding Session, June 23, 2000
Lyndon B. Johnson Space Center, Houston, Texas

Participants:

Bonnie J. Dunbar
National Aeronautics and Space Administration

Larry DeLucas
University of Alabama – Birmingham

Richard Fullerton
National Aeronautics and Space Administration

Dennis Morrison
National Aeronautics and Space Administration

Neil Pellis
National Aeronautics and Space Administration

Fifth Fact-Finding Session, November 8, 2000
U.S. Army Edgewood Chemical Biological Center
Aberdeen Proving Ground, Maryland

Participants:

James J. Valdes
U.S. Army Soldier and Biological Chemical Command

Kevin O'Connell
U.S. Army Edgewood Chemical Biological Center

Jennifer Sekowski
U.S. Army Edgewood Chemical Biological Center

Joe DeFrank
U.S. Army Edgewood Chemical Biological Center

Erica Valdes
U.S. Army Edgewood Chemical Biological Center

Jun Park
Geo-Centers, Inc., U.S. Army Edgewood Chemical Biological Center

Akbar Khan
U.S. Army Edgewood Chemical Biological Center

Sarah Cork
Geo-Centers, Inc., U.S. Army Edgewood Chemical Biological Center

Roy Thompson
U.S. Army Edgewood Chemical Biological Center

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Appendix C

Chemical Sensing Using MEMS Devices

Of all the microelectromechanical systems (MEMS) devices being developed, chemical sensors are probably the most difficult but also the most needed (Wise, 1996). Whereas mechanical sensors commonly provide levels of resolution and accuracy exceeding 12 bits (0.02 percent), the demands placed on MEMS-based chemical sensors are more exacting and, in some instances, may not be realizable. Indeed, chemical sensors must provide high sensitivity, stability, selectivity, specificity, and speed.

Some of the MEMS devices being explored do not have sufficient sensitivity or dynamic range for use in the field, especially over extended periods of time. Worse still, most chemical sensors are not stable and drift randomly at levels that are significant with respect to the overall dynamic range. Many also lack selectivity and respond similarly to a number of agents, only some of which may be hazardous. They also lack specificity and are unable to pick one (harmful) gas from other (benign) gases that may be present. Finally, response times are usually many seconds and can be many minutes, far too slow for wearable use. The human body would, unfortunately, respond faster.

Chemical sensors must be very sensitive (at the parts-per-billion level), stable, and robust enough for use in the field, selective and specific to gases of interest (yet generic in approach), and fast enough to protect nearby personnel. The sensor should also be low enough in operating power to be deployed in the field in a small, lightweight package (similar in size to a credit card or wristwatch).

Several different types of MEMS devices are being explored as chemical sensors: devices based on gas absorption in bulk films; devices based on surface adsorption and microcalorimetry; conductivity-based devices ("microhotplates"); and full microinstruments, such as integrated gas-chromatography systems. Devices based on the selective

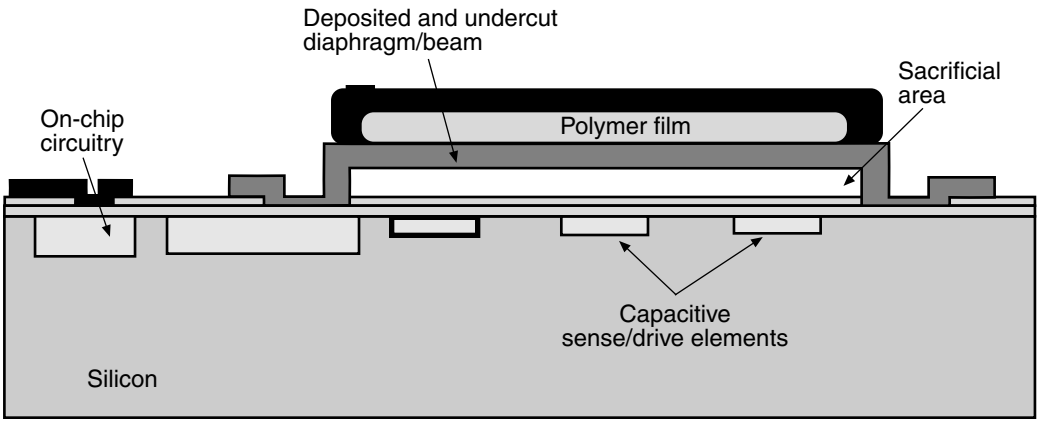

FIGURE C-1 Cross-section of a vibrating-beam vapor sensor based on surface micromachining. The polymer film absorbs a specific vapor, changing the film mass and the vibration characteristics of the beam.

Source: Courtesy Dr. Kensall Wise, University of Michigan.

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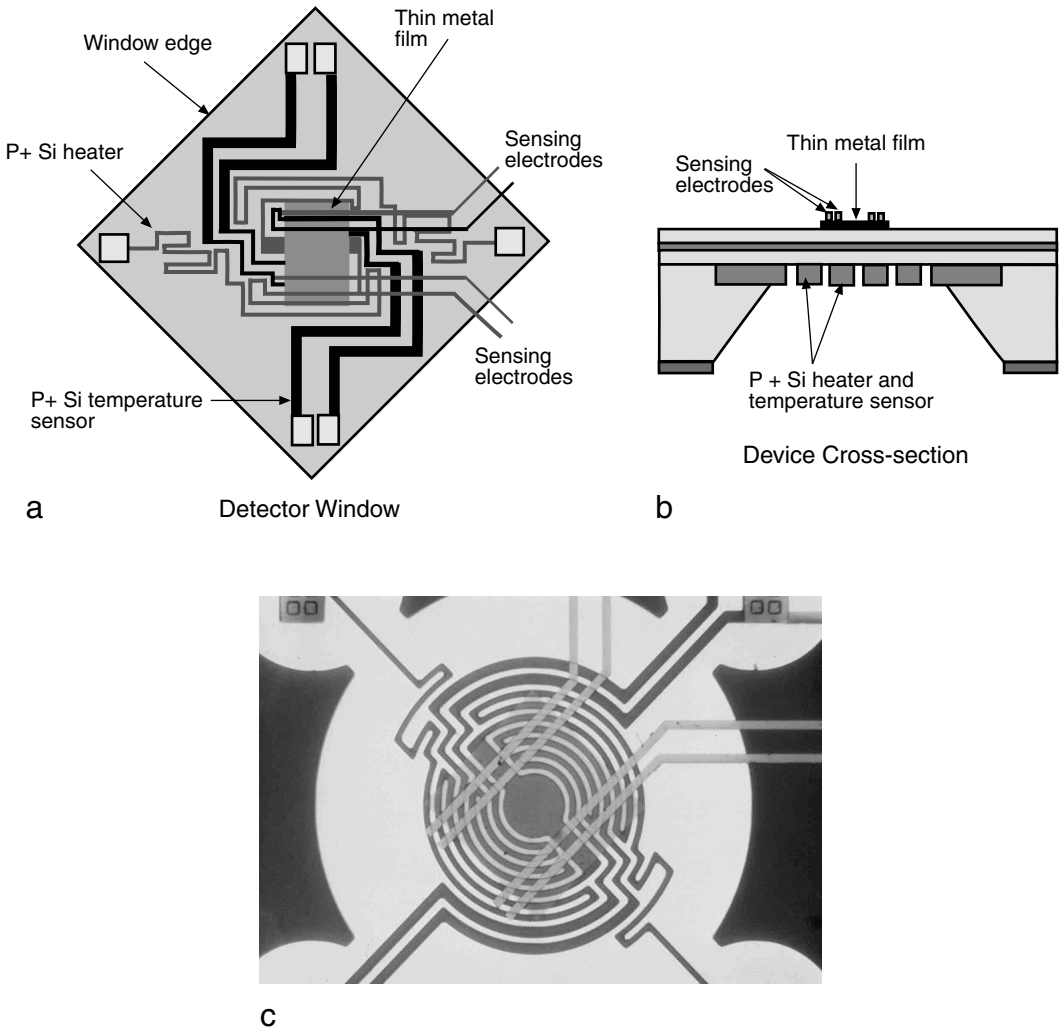

FIGURE C-2 Top view (A), cross-section (B), and photograph (C) of a "micro-hot-plate" gas sensor. A dielectric window thermally isolates a heater and detecting film from the rest of the chip. As gas adsorbs on the detecting film, the change in film conductivity can be detected electronically. The window shown here is 1mm in diameter.

Source: Courtesy of Dr. Kensall Wise, University of Michigan. Part C reprinted with permission from Najafi et al., 1994. Copyright 1994, IEEE Transactions on Electron Devices.

absorption of the chemical of interest in a thin bulk film usually detect the increase in mass that results from that absorption (Harsanyi, 1995). An early version of such a device was the vapor sensor of Howe (Howe and Muller, 1986) (see Figure C-1). It consists of a beam (typically polysilicon) which is deposited over a sacrificial layer (often phosphosilicate glass [PSG]). The beam is coated with a polymer that selectively absorbs the gas of interest; the sacrificial layer is then etched away, releasing the beam. In operation, the beam is driven electrostatically across the 1 to 2μ air gap left by removing the sacrificial layer, and the beam vibration is sensed capacitively. As the mass of the polymer changes, the resonance frequency and vibration amplitude shift, providing a measure of the vapor concentration. Such devices can be relatively sensitive (the frequency shift due to saturated xylene vapor in the Howe device was about 0.3Hz/ppm), but most polymers are not very selective or very specific. Temperature sensitivity and stress effects are also difficult to predict and control. Time responses are set by diffusion into the sensing film and are measured in tens of seconds to minutes. (The response time of the Howe device was about 7 min.)

Surface acoustic wave (SAW) devices (Ballantine et al., 1997; Ricco et al., 1998) represent another implementation of structures in which changes in absorptive mass lead to a frequency shift in a surface wave, which can be detected

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differentially. Excellent work on SAWs has been carried out at Sandia National Laboratories. Chemiresistor arrays are another sensing approach worth watching that is currently being explored (Hughes et al., 1998).

Devices based on microcalorimetry (developed by IBM) have demonstrated that it is feasible to measure very small quantities of specific gases (Gimzewski et al., 1994). Using gold-coated, silicon cantilever arrays, the stress-induced bending of the cantilevers with molecular adsorption can be detected optically, and arrays of such structures are being developed as the equivalent of an artificial nose (Lang et al., 1999). By operating the sensor in several different modes, including cantilever bending (static) or resonance frequency shift tracking (dynamic), "orthogonal" responses can be obtained to distinguish between similar compounds. These devices are still in development but are worth watching for near-term (less than five years) commercial availability. Specificity and robustness in the field remain areas of concern for military applications.

Conductivity-based devices rely on the change in the resistance of a thin conducting film with adsorption or absorption of specific gases (Najafi et al., 1994). If metal- or metal-oxide-detecting films are used, they are supported on dielectric platforms that are thermally isolated from the body of the chip and its package (see Figure C-2). Because these devices typically operate at elevated temperatures (200–400°C), the on-state power requirements are tens of milliwatts or more for a single element. Response times are a few seconds. Conductivity-based devices can be very useful for detecting trace quantities of gases (a few parts per million or less), but they become saturated when a few monolayers of surface coverage are obtained. Even if the power requirements for such devices are acceptable, high specificity and selectivity are difficult to obtain. Selectively permeable membranes and/or detector arrays coated with many different films are being explored to improve performance. Microprocessor-based detection algorithms and/or neural nets to deconvolve the array signature into information on specific gases in a gaseous mixture will then have to be developed (Osbourn et al., 1994).

Temperature-programmed desorption can also be very helpful in identifying different species. Although it is possible to "program" completed arrays of microhot-plates with different detecting films using chemical vapor deposition on selectively heated dielectric windows, achieving acceptable selectivity, stability, speed, and sensitivity with these devices remains a formidable challenge (Majoo et al., 1995). Unless there are breakthroughs in detecting

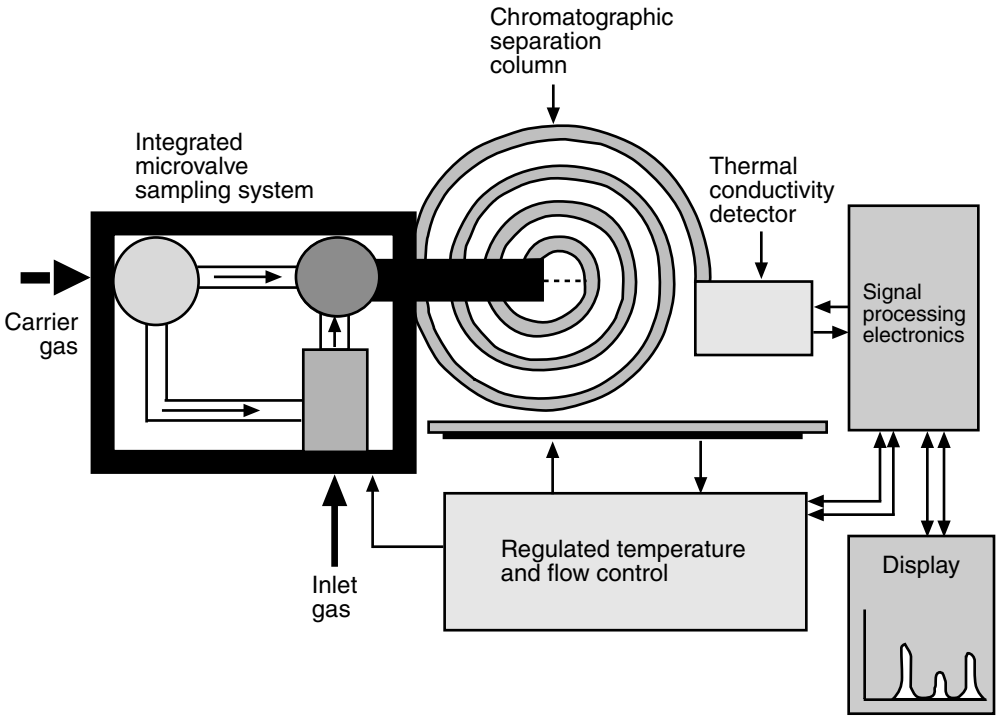

FIGURE C-3 Block diagram of a monolithic gas chromatography system. Microvalves and microfluidics are major areas of interest in MEMS today.

Source: Courtesy of Dr. Kensall Wise, University of Michigan.

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films, these devices are unlikely to meet the requirements for detecting biohazards. Intermediate bioreceptors may make such breakthroughs possible. Thus, active-surface chemistries may greatly expand the utility of chip-based sensing platforms for both chemical and biological agents.

Chromatography, the most common laboratory technique for analyzing gaseous mixtures, is based on the fact that different molecular species spend different amounts of time adsorbed to surfaces as a sample of the gas is passed through a long tube (column). Thus, if a sample containing a mixture of gases is injected at one end of a long tube, the gases will emerge from the distant end of the tube separated in time. By calibrating the column delays for different known gases, the constituents of an unknown mixture can be identified. Chromatographic devices are sensitive, stable, specific, and selective.

Sensitivity can be enhanced by slowly absorbing the gas mixture of interest in a substance in front of the column (i.e., a preconcentrator) and then rapidly releasing it thermally into the column. If these systems can be miniaturized, they can also be relatively fast. Tabletop gas chromatographs (GCs) have column delays of several tens of minutes, but integrated miniature GCs, first proposed in 1971 and first reported by Terry et al. in 1979, can work much faster (a few minutes or less) (see Figure C-3). However, these microinstruments require an integrated gas-sampling system, which is only now becoming feasible. They also require a source of carrier gas or a pumping system, which has not yet been realized at the microscale.

Miniature GCs are now under development at Sandia and Lawrence Livermore National Laboratories. Calculations support the feasibility of achieving a sensitivity of 10 to 100ppb in a miniature GC, detecting atmospheric pollutants, and operating at less than 2mW in as little as 2 cm^3 of space. This technology has, perhaps, a 10-year horizon to availability. A minaturized system could detect a variety of hazardous gases and could be deployed as a wrist-mounted sensor in the field. Detecting multiple biological agents is much harder, however; it will require a true chemical laboratory-on-a-chip based on microfluidics.

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Appendix D

Vaccination

Vaccination is the deliberate immunization of an organism against infection by a disease agent. It can also mean the immunization of a person against any agent capable of provoking an immune response. The agent provoking the response can be an infectious organism, but it can also be a medium-sized molecule (i.e., a protein toxin) or part of a protein from one's own body (e.g., stimulating an immune response to a tumor). Anticipated advances in cell biology, immunology, molecular genetics, genomics, and cellular immunity will greatly accelerate the production of cheap, safe, effective vaccines.

Vaccines prepare the immune system to recognize and attack invaders or antigens. Once an organism has been vaccinated, it becomes immune because it contains populations of cells carrying molecules on their surfaces that recognize particular antigen molecules produced by, or part of, an infectious agent. Antigens are frequently, but not always, proteins encoded by the genome of the infectious agent; they can also be other molecules, such as complex carbohydrates.

IMMUNE RESPONSE

The technology of vaccination was worked out considerably before any of the underlying science was understood. In the late nineteenth century, the observation that people who had been infected or exposed to a disease agent were subsequently immune to it was extended by rigorous experimentation. The panoply of responses to an invader became known as the *immune response*. The fact that the immune response is faster and stronger on reinfection than on first infection, meaning that the organism "remembers" that it has once been infected and remembers how to deal with it, became known as *immunological memory*. The organismic system responsible for the immune response became known as the *immune system*.

The different types of (white) cells that orchestrate and execute this complex behavior circulate freely in the blood, where they are relatively easy to get at; for this and other reasons, advances in understanding the immune response in the twentieth century were breathtaking. The exponential increase in scientific knowledge is playing out in sophisticated methods (tactics) of exploiting the immune system to meet specific goals.

In the textbook picture, the immune system is divided into two arms, one that uses antibodies to recognize and help destroy intruders (also called B-cell arm, humoral immunity, and serum immunity) and one that uses cells (also called T-cell arm and cell-based immunity). A stripped down version of the canonical picture of the natural history of a "typical" immune response is described below.

An invader, let us say a bacterium or a virus, replicates, let us say in the blood (or, if a virus, in the cells of the blood). The invading organism is made up of molecules, lipids, nucleic acids (e.g., DNA) and proteins. The molecules, and parts of molecules, that are recognizable by the immune system are referred to as *antigens*.

B-Cell Arm

Some of the invading organisms circulate in the blood and lymph. In the spleen, antigens on some of the viruses match (are recognized by) antibodies on the surface of particular B-cells. When cells that bear these antibodies start dividing, they create a *clone* of cells descended from the founder cell that recognized the antigen (*clonal selection*). Many of these daughter cells begin to make mutant antibodies, some of which bind more tightly to the antigen. Those that do proliferate more. Thus, the longer the antigen is around, the more cells make antibodies that bind it strongly. Later, cells that carry the tight-binding antibodies on their surfaces rewire themselves internally so that the antibodies are secreted into the blood and lymph.

If the invader is a virus, circulating antibodies destroy the virus by binding to it and gunking it up into large complexes (*immune complexes*) that are cleared from the blood. If the invader is a bacterium, complex formation can happen as

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well, but, in addition, the binding of the antibody to the large bacterium triggers a complex series of events, all caused by proteins acting on other proteins (the *complement cascade*). The call-down of the complement cascade results in the bacterium being punched full of holes, which, as ions leak in and leak out, kills it.

After the infection is over, a small population of B-cells rewires itself to express on its surface the antibodies that worked best. If the invader returns, these cells (*memory cells*) proliferate immediately. On the second pass, therefore, the process does not start anew but begins at the end of the cycle.

T-Cell Arm

Some of the invading bacterial and viral particles are ingested by *antigen-presenting cells (APCs)*, such as macrophages and dendritic cells, which in turn chew up the viral proteins and display pieces of them on their surfaces. The APCs collide with other cells, including one flavor of T-cells, called *T-helpers* (actually Ths, of the *Th1 subclass*, so *Th1 cells)*. Some Th1s have, on their surfaces, molecules that happen to bind to one or another piece of chewed up viral protein (the antigen). The helpers make soluble proteins (e.g., interleukin 2, or IL-2) that stimulate the other main class of T-cells, called *killers* (*Tk*), also known as *cytotoxic T-cells* (*Tc*), to divide.

If a cell is infected with a virus, some of the proteins it makes are encoded by the virus. The cell displays pieces of these viral proteins (as it displays pieces of its own proteins) nestled in a cleft on a particular kind of molecule called *HLA class I*. Tk cells have on their surfaces molecules called *T-cell receptors* (*TCRs*). TCRs on different Tk cells come in many different shapes, and some Tks have TCRs that recognize and bind to the complex between HLA class I and viral proteins found on the surface of the virally infected cell. When a Tk has bound to a cell, it is said to be *antigen activated*. In the presence of IL-2 from the Th1 cells, these antigen-activated Tk cells proliferate and turn into active killers.

When an active killer runs into a foreign cell with HLA on its surface that it recognizes, it binds to it, punches holes in it, and kills it. When the immune response is going well, the killer can recognize the virally infected cell and destroy it before progeny viruses are produced. Antigen-activated Tks tend to persist. On reinfection, in the presence of IL-2, they proliferate again, and the reponse picks up at the end of this stage of the process.

After vaccination, the organism that has been vaccinated is said to be *immune*; that is, it cannot be easily reinfected by the disease agent. Immunity means it now contains populations of cells that carry on their surfaces molecules that recognize particular molecules (*antigens*) that are made by, and are part of, the infectious agent and that are recognized by the immune system. That is, antigens are defined operationally as those molecules or parts of molecules to which the immune system responds. Antigens are frequently but not always proteins encoded by the genome of the infectious agent; they can also be other molecules, such as complex carbohydrates.

It is perfectly appropriate to think of the immune response as an amazingly sophisticated identification-of-friend-or-foe (IFF) defense mechanism. In this view, the immune system represents a paradigm of a distributed network that can perform a sophisticated task (recognition of an intruder, decision making, execution). The response is robust, redundant, and nonhierarchical.

Communications among components of the immune system are of two different, low-bandwidth types: (1) cell-cell touching, which can convey pieces of molecules (high-semantic content) and (2) secretion of proteins (~100 different proteins in all), which are received by cells if the receiver cell has the right receptor and if concentration of the secreted protein is high enough. Although local cells may be more affected, the communication is nondirectional spatially; that is, components of the system exist in no known, or fixed, orientation or relationship to one another. This combination of high and low bandwith, slow signaling, decentralized decision making, and timely, accurate response is unparalleled in the design and manufacturing world.

VACCINE DEVELOPMENT AND PRODUCTION

Recent developments in cell biology, immunology, molecular genetics, and genomics relevant to the Army are likely to lead to the development of less complex vaccine products (e.g., recombinant viral proteins or DNA encoding for these proteins), the generation of protective immune responses against a wide range of pathogens, and the creation of well-characterized products manufactured by robust, fast, inexpensive process technologies.

Generally, six types of vaccines are used today:

1. killed infectious organisms (inactivated vaccines)
2. naturally occurring infectious organisms that are closely related to the pathogen (e.g., the cowpox virus used in vaccinations against smallpox is closely related to smallpox but does not cause the disease in humans)
3. live infectious organisms that have been treated, or mutated, to be less virulent (live attenuated vaccines)
4. subunit vaccines
5. cell-based and virus-based vaccines
6. DNA vaccines

The impacts of developments in biotechnology on types 3, 4, 5, and 6 are described below. The impact on *adjuvants*, substances used in vaccines to increase the strength of an immune response, are also described. The final section is a discussion of how recent developments in biotechnology could open the way to new ways of conferring immunity.

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Live Attenuated Vaccines

Selected attenuated vaccines are conventionally generated by growing a bacterium or virus over many generations in a host different from the organism to be vaccinated. Because growth in a different host organism exposes the pathogen to a different selective environment, the pathogen undergoes a number of mutations that adapt it for the new host and make it less fit in its normal host. For example, to generate a virus against influenza, the new flu strain is grown for many generations in chicken eggs. Whatever helps the virus grow best in chicken eggs is not the same as whatever helps it grow best in humans, so it is less fit in humans and less able to cause disease in the original host. These debilitated or weakened mutants are selected for the vaccine.

Recently, at least for viruses, this classical approach to selecting mutant viruses has been supplemented by the deliberate introduction of random or directed mutations. Selecting viruses that are, for example, temperature-sensitive results in viruses that are less able to grow well in the host. Directed mutagenesis (mutation development) of genes in viruses that are antigenic is another widely used technique. A gene that is useful but not necessary for growth, for example, can be removed and tested to see if infection with the mutant virus confers immunity. These obvious molecular tactics could be extended to bacterial pathogens.

Subunit Vaccines

In the last century, biology was focused on individual molecules responsible for disease and immunity. Today, the vaccine industry has largely moved beyond the use of killed or live attenuated whole organisms to vaccines that contain key molecules sufficient to confer immunity. Genomics is moving the science and practice of vaccination down to the level of the genes that confer immunity.

Most modern vaccines are subunit vaccines, that is, vaccines that contain one or more molecules or parts of molecules that carry the immunological properties of an organism, which in turn can elicit the immune response. Generically, the process of generating a subunit vaccine for an organism involves a survey of organisms that had been infected with the infectious agent and recovered to determine which antigens provoked an immune response. Those antigens, typically proteins, are then manufactured, typically as recombinant proteins expressed in bacteria, yeast, or cultured animal cells. The recombinant proteins are typically mixed with an adjuvant and injected into test animals to determine if they confer immunity. Based on these animal studies, the mix of antigens and adjuvants can be fine tuned. Eventually, trials are conducted in humans.

Recombinant DNA technology and immunology were successfully used in the search for the surface antigen of an invading organism and the development of a recombinant vaccine for hepatitis B. The tools and methodologies for this concept are well proven and could be easily extended to other systems.

Moving from flu vaccine in chicken eggs or vaccinia from cow pustules, to cultures of pure organisms *in vitro* to recombinant proteins from those organisms, leads to well defined and pure vaccines. Less complex vaccine products, such as recombinant proteins and DNA, are generally easier to manufacture and characterize. Therefore, reproducible, consistent processes might be developed for producing them at reasonable cost and on reasonable schedules. For example, the United States has far more cell culture capacity for producing most recombinant protein and plasmid DNA products (biosafety level GLSP/BL-1 cell-culture capacity) than capacity for producing vaccines from pathogenic infectious agents (BL-2+ cell culture capacity).

Given the complexities associated with supplying vaccines for the Army, manufacturing strategy should be considered at an early stage in their development. Genomic techniques promise to simplify the task of identifying candidate antigenic molecules. Antigenic proteins tend to be on the surface of the bacterium, and the set of proteins expressed on the surface when growing inside cells is likely to contain almost all of the proteins made by the bacterium that causes the immune response. Similarly, antigens are likely to be found in the set of proteins secreted by the pathogen and among the genes that are transcribed in the messenger RNA during the course of an infection. Consider a bacterium that grows in human cells, for example. Inspection of its genome sequence is now sufficient to identify the protein molecules encoded by its genome that might be expressed on its surface. The subset of those proteins that are expressed when the bacterium grows inside cells can be identified by mRNA expression analysis, which should give the most likely antigen candidates. As always, improvements in underlying technologies will accelerate these analyses. For example, sequencing of single strands of DNA through nanopores may make possible the sequencing of entire bacterial genomes at much higher speeds.

Cell-Based and Virus-Based Vaccines

In cell-based vaccines, nondisease-causing cells or viruses are genetically altered to display antigenic molecules, typically antigenic proteins, derived from an infectious organism. The displaying cells can, for example, be beneficial bacteria that usually inhabit the respiratory tract or gut, or even somewhat pathogenic bacteria that cause a mild infection and displace existing flora for a few days. These altered cells are used as the vaccine to elicit the immune response.

Despite some research in this area, much of which was funded by the Defense Advanced Research Projects Agency (DARPA) Unconventional Pathogens Countermeasures Program, no cell-based vaccines have been approved for use against infectious diseases. However, they are used in cancer therapy for myelomas. Nowadays it is reasonably

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common to take tumor cells from a patient, grow them *in vitro*, and reinfuse them into the patient in an attempt to cause the immune system to react against the cancer. The effectiveness of these vaccines can be increased by introducing into the tumor cells DNA that directs the synthesis of proteins that increase the antigenicity of the cancer cell. This approach has been very successful against adult-onset leukemia and lymphoma, curing 50 percent to 80 percent of patients who would otherwise have died. However, trials have been limited to dozens of persons.

Another promising approach is the use of viral vectors (engineered viruses) to deliver antigens that confer immunity against multiple infectious diseases in one vaccine. For example, consider vaccinia, the cowpox virus used to immunize against smallpox. Vaccinia, a double-stranded DNA virus with a large genome, contains enough dispensable DNA in principle to direct the synthesis of numerous foreign proteins. That is, pieces of vaccinia DNA can be cut out and replaced with other DNA that encodes other antigens. Vaccinia derivatives were produced during the 1980s that could direct the synthesis of foreign proteins based on this principle; one could imagine using vaccinia or other vectors that carry multiple antigens to develop a single vaccine that would confer immunity against multiple infections at a very low cost per dose (e.g., $0.25).

DNA Vaccines

DNA vaccines are another promising new type of vaccination. Because DNA can be manipulated much more easily than proteins or living organisms, the development of DNA vaccines raises the possibility that new vaccines, or vaccines against new organisms, could be generated and distributed quickly—within weeks of identifying a pathogenic organism.

In one version of this approach (developed at the University of Texas Southwestern Medical Center in Dallas), fragments of DNA that direct the synthesis of protein antigens specific to that organism are generated by polymerase chain reaction (PCR) from the genome of the organism. The assembled piece of DNA is mixed with other DNA constructs that direct the synthesis of cytokines or proteins (e.g., interleukin-12) that stimulate the immune response. Gold spheres with slight surface roughness are mixed with the DNA, and the spheres are shot (using a *gene gun*) into the skin of the animal being immunized. Some of the DNA is taken up by dendritic cells in the skin and presented to the immune system, which initiates the process leading to an immune response.

The ultimate promise of this technology is the deconvolution of a pool of genes in a whole-genome, shotgun approach in a few weeks, as opposed to years. To identify the particular genes that would confer immunity, the researcher could take all of the potential antigen-encoding DNA, break it into pieces, amplify it using PCR, express it in mice, and find the products that render the mouse immune to infection. (The limitation is that mice are not the same as people and are not susceptible to all of the same diseases.)

Conceptually, the information-processing part of generating DNA vaccines is identical to the technique used for subunit vaccines: the antigenic molecules (here, exclusively proteins) are identified and tested in animals. The pieces of DNA that go into the vaccine must then be specified. But because it is so much easier to manipulate DNA than produce numerous different recombinant proteins, it becomes cost effective to pursue tactics such as immunizing different animals with all possible different genes or combinations of genes (*pooling* approaches), identifying which animals become immune, and combining the genes that work in a single vaccine.

DARPA has invested heavily in this technology. In fact, the United States' rudimentary, experimental surge capability to make vaccines is almost entirely attributable to the DOD research. DNA vaccines are now being pursued by many U.S. biotechnology and pharmaceutical companies. The Army should keep abreast of this research.

ADJUVANTS

An *adjuvant* is a substance that increases the immunogenicity of an antigen. Adjuvants work by, for example, increasing the number of APCs (antigen-presenting cells) that migrate to the site of the vaccination or by emulsifying the antigenic proteins in the vaccine preparation so that they are partly unfolded and can be more easily recognized and ingested by the APCs. In some ways, the use of adjuvants is a century-old technology. However, recent advances in the understanding of the immune system have brought to the attention of vaccine makers molecules (e.g., interleukin-12) that can stimulate the immune system by well understood mechanisms. The Army's vaccine program has a longstanding interest in adjuvants and is already following these developments closely.

IMMUNITY BY OTHER MEANS

Two other means of conferring immunity could be affected by emerging biotechnologies: passive immunization and innate immunity.

Passive Immunization

An injection of antibodies (IgG) from an immune individual confers a transient, IgG-based immunity to the disease agent as long as those antibodies continue to circulate in the blood of the person receiving the injection. Because there are no memory cells in the immunized individual, protection lasts only as long as the injected antibodies continue to circulate (typically, weeks). Therefore, the immunity conferred by this technique is called *passive immunity*. At the

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start of the twentieth century, sera that confer passive immunity to infectious diseases, such as diphtheria, were generated by injecting antigens into horses and purifying the IgG fraction from their serum. This technique is still sometimes used. For example, a person traveling to a country where a disease, such as hepatitis A, is endemic might receive an injection of gamma globulin made from a human who has recovered from the disease.

With advances in understanding, new techniques are being developed. A number of means are now used to isolate genes that encode human (or humanized) antibodies against any antigen. Once the gene has been isolated, the antibodies can be produced *in vitro*, in cell culture, or in milk, eggs, or even plants. Both for diagnosis and for prophylaxis (i.e., vaccination), the United States should develop a surge capacity to produce antibodies. DARPA has at least considered funding such work, and the Army should support it.

Stimulating Innate Immunity

The complex B-cell and T-cell arms of the immune system enable the organism to become immune to an infectious agent after its immune system is exposed to the agent. Organisms also possess *innate immunity*, which enables them to respond to infectious bacteria and viruses to which they have not been previously exposed.

Some mechanisms of innate immunity predate the modern immune system. For example, cells have receptors for types of molecules found on bacterial surfaces, and when those receptors are bound, the cell becomes more resistant to infection. Much more recently, vertebrates have evolved other pathways. For example, *interferons* cause most cells to initiate responses that make it more difficult for viruses to grow in them. There are also "natural killer" (NK) cells that can rapidly mobilize and attack foreign cells without having been educated as to the nature of the foreign antigens. The complex of cytokines that choreographs and directs the NK response is increasingly well understood, but the mechanism(s) by which NKs recognize cells as foreign is not. In the next 25 years, more will certainly be learned about the molecular nature of these phenomena; eventually it will be possible to manipulate these mechanisms to confer immunity or to block the progress of an infection once it has started. These techniques are likely to be too experimental to be pursued commercially. DARPA is now one of the lead agencies funding this promising research, and the Army should follow developments closely.

GLOBAL IMPACT OF ARMY DEVELOPMENTS

The Army wishes to be able to protect its forces against disease no matter where those forces may be deployed. By contrast, the pharmaceutical industry requires the projection of peak annual sales on the scale of hundreds of millions of dollars per year before deciding that a drug will be sufficiently profitable to justify its commercialization. The markets that can bear this expense are the approximately one billion people of the affluent areas of Europe, Japan, East Asia, and North and South America. Because commercial

BOX D-1
Malaria

Malaria is a global public health problem. A staggering 40 percent of the world population lives in areas where malaria is transmitted, resulting annually in an estimated 280 million to 1 billion cases, and 1 to 3 million deaths (WHO, 1999). The most severely affected populations are young children and pregnant women and infants.

The threat of malaria is not restricted to people living in malaria-endemic areas. Military personnel, State Department personnel, and travelers who visit these areas are also at risk. Infection with *Plasmodium falciparum*, the most virulent human-infective parasite, can be fatal to nonimmune individuals. In addition, in many areas the parasite is becoming resistant to the current prophylactic antimalarial drugs. The development of new antimalarial drugs is lagging far behind the development and spread of resistant parasites. Mosquitoes, the insect vector for malaria, are also developing resistance to insecticides. Even if effective, insecticides are too costly to be a viable control measure in malaria-burdened nations.

An obvious solution for the failure of antimalarial drugs and the difficulties inherent in large-scale mosquito control is the development of an inexpensive vaccine that could be used to protect both travelers and indigenous people in malarious areas. In recent years, several promising vaccine candidates have emerged, but progress has been slow. A major impediment has been lack of sufficient funding to support malaria research. In 1999, for example, the National Institutes of Health, the leading funding body for research in infectious diseases in the world, allocated $24 million for extramural research on malaria. This level of funding falls far short of the estimated $1.6 billion some believe is necessary (CID, 2000).

Research should be focused on an in-depth investigation of the complex relationships between the malarial parasite, the human host and the mosquito vector, and the nature of the immune response that must be elicited by a vaccine. The natural development of immunity to malaria in exposed people, such as children who survive multiple infections in their first few years of life, and the alteration of immunity experienced by pregnant women, should be explored. How these immunological changes will affect the delivery and efficacy of a malaria vaccine will also have to be investigated.

Malaria is not only a problem of developing nations. It also has important implications for the continued development and prosperity of all nations. Concerted global efforts, starting with vigorous research and culminating with the delivery of effective, sustainable, affordable interventions for everyone at risk would have large-scale benefits.

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small-molecule drug development has been focused on specific targets, it is unlikely that therapeutics will emerge for unrelated diseases. Even research on diseases common to rich and poor countries typically target the strains of the disease found in rich countries. Since Army forces are far more likely to be deployed to poorer countries, the Army should identify ongoing developments that it could use to leverage future development of therapeutics against the infectious diseases found in poorer countries.

For years, the Army has been one of the main promoters of the development of drugs against diseases endemic to poor countries, including malaria, which is responsible for 1 million to 3 million deaths annually (see Box D-1). In the future, the Army could have a considerable impact on the treatment of bacterial and protozoan parasitic diseases and viral infections that are prevalent in the relatively undeveloped areas of the world. By 2020, an estimated 7 billion to 8 billion people will live in less affluent areas of the world. Because it must be prepared to deploy forces in these areas, the Army should continue to remain closely involved in the development of therapeutics that could not only protect U.S. forces, but also contribute to world health.

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