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LAWRENCE
LIVERMORE
LABORATORY

STATE OF THE LABORATORY

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ABOUT THE COVER

This aerial photo is from the southwest corner of the LLL site, which is about one mile square and was originally a Naval Air Training Station during World War II. Many of the original barracks buildings (right) are still used, along with temporary trailers, to house some 1200 employees. Prominent site features include a large six-story building (right background) built in the early 1950's, which would house the proposed MX experiment, and the seven-story building (center foreground) built in the late 1960's, which accommodates several hundred nuclear-weapons design people. Just out of range, at the far left, is the new 25-TW High Energy Laser Facility under construction immediately north of the Laser Fusion Laboratory.

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The short items on these pages announce recent developments of importance. Some of these items may be amplified in future issues; none of this material is reported elsewhere in this issue.

TOPPING CYCLE DEVELOPMENT STARTED

We are beginning work on a topping cycle to improve the efficiency of coal-fired steam-generating plants. Our first step will be to evaluate the Lysholm expander, a rotary engine that seems potentially capable of efficiently using the high-temperature combustion products from burning coal. Improved efficiency of coal-fired systems is important because, in view of the high cost of imported oil and the slow pace of nuclear power growth, we can expect to be using much more coal, at least for the next few decades.

The best present coal-fired power plants operate at 40% thermal efficiency with a peak steam temperature of 550°C. The Lysholm expander would operate with an inlet gas temperature of 1400°C and discharge hot gas at 800°C to a boiler to raise steam to the standard 550°C level. The overall efficiency of such a combined cycle would be higher than 50%, yielding more than 25% extra energy from each unit of coal.

Improved coal-fired power plant efficiency translates directly into major environmental benefits. It means less coal burned per kilowatt, therefore less air pollution, less strip mining, and less waste heat discharged to the environment. It could also provide major economic benefits in terms of lower power costs and less dependence on foreign fuel sources.

Several different coal-fueled topping-cycle projects are under way in this country under sponsorship by ERDA, other agencies, and industry. The LLL effort involves preliminary design and testing of expanders, development of suitable ceramic materials in the silicon nitride and silicon carbide families, studies of the erosion and corrosion effects of coal condensation products on these materials in the expander application, and computer simulation studies.

The Lysholm engine's chief competitor is the gas turbine. Gas turbine design has made great strides recently and is approaching the demonstration stage. Present turbines have a major drawback, however; they cannot tolerate the fly ash that always comes from burning coal. Such a gas-turbine topping cycle is practical, therefore, only if the coal is prepurified, either by gasification or by solvent extraction. However, the extra expense of this purification step makes the topping cycle far less attractive economically.

Research is proceeding on ways to make turbine blades that will resist fly-ash erosion, e.g., by making them out of super-hard ceramics. However, the brittleness and high thermal expansion of these ceramics make their manufacture into thin, long turbine blades extremely difficult.

The Lysholm engine, by contrast, should be extremely tolerant of fly ash. There are no high-speed, high-stress components. Two massive, intermeshed spiral rollers are the only moving parts. Fly ash may even improve the Lysholm engine's performance by reducing leakage through the close-tolerance gaps between the rollers and the outer casing.

VISITOR'S CENTER NEARING COMPLETION

LLL averages some 1500 visitors monthly, nearly all on business. Until recently, almost all public sightseeing visits were funneled into one of the occasional visitors' days: Family Day, Youth Science Day, etc.

Now, however, the first unit of a three-module Visitor's Center is in final stages of completion. This 230-m² (2500-ft²) building will house displays on current LLL projects, a small temporary theater for showing a multimedia film on directions the Laboratory is taking, and a "time-line" photographic display of LLL history. The Center will be open and manned five days a week: Wednesday through Sunday. Multilingual tour guides will be available.

The first unit is scheduled for formal opening in late July. This will be followed next year by addition of a permanent theater and an exhibit building for expanded displays including "hands on" exhibits and demonstrations related to LLL projects.

First unit of LLL Visitor's Center is now nearing completion.

PERMANENT QUARTERS FOR DAS

The Department of Applied Science (DAS) will move into a new, permanent classroom building here this Fall, roughly doubling classroom and office space.

The DAS, founded in 1963 and quartered on LLL grounds, is administered by the University of California (Davis) College of Engineering. The University has awarded 106 Master's and 84 Ph.D. degrees since its inception. Many graduates have joined the LLL staff.

Close relationships are maintained with the Laboratory. Approximately 25 LLL scientists and engineers now serve as DAS faculty or lecturers. Many Laboratory personnel (37 of the present 91 students) also take advantage of the close-at-hand DAS educational opportunities.

New classroom building for Department of Applied Science will open this fall.

AUTOMOTIVE COMBUSTION STUDIES UNDER WAY

Improved automotive engine efficiency and reduced undesirable emissions are the targets of a new research effort now under way at LLL.

The effort is primarily computational, focusing both on detailed combustion physics and chemistry and on generalized models computer simulations of

combustion systems, especially the internal combustion engine. Some experimentation will be involved, as well as development of new diagnostic and measurement techniques. It is believed that a more precise understanding of combustion physics, turbulence within combustion cylinders, heat losses through cylinder walls, fuel-droplet motion and vaporization characteristics, and other such phenomena may lead to new methods of improving engine design, operation, and efficiency.

The program will emphasize computational techniques that can be transferred to industry for use in the private sector. Funding is established at \$60,000 for the balance of the current fiscal year. The effort is expected to continue at least through FY 1977.

NRC FUNDS NUCLEAR WASTE MANAGEMENT STUDY

LLL has been funded by the U.S. Nuclear Regulatory Commission (NRC) to provide technical support in the areas of nuclear waste regulations, criteria, and standards. The LLL effort will emphasize a multidisciplinary approach to problems of special interest to NRC, including routine waste management operations, the effects of various process options on a disposal system's performance during active disposal, and the results of geological and chemical interactions during long-term storage.

Funding is set at \$200,000 for the balance of FY 76 and FY 76T.

Our immediate objectives between now and September 30 include assembling the multidisciplinary team and providing, on relatively short notice, wide-ranging technical support to NRC on waste management problems. We intend to organize a comprehensive data base, determine a technical basis for performance criteria on solidified, highly radioactive wastes, and develop a meaningful classification scheme for nuclear wastes. The latter may serve as a regulatory basis for specifying waste management and disposal criteria.

The B77 (top) is a new strategic bomb being developed for the B-1 bomber and other strategic aircraft. The B77 can be released from a bomber flying at supersonic speeds at a 100-ft altitude; a lifting parachute and rugged design provide the capability for laydown delivery against hard, irregular targets. This delivery mode greatly increases aircraft survivability. The B77's insensitive high explosive also enhances safety in accidents. The W79 is a new artillery shell for the Army's 8-in. cannon (bottom). It will contribute to tactical deterrence by providing improved simplicity, control, and accuracy, increased range, and greater utility on the tactical battlefield.

STATE OF THE LABORATORY

ACCOMPLISHMENTS AND TRENDS AT LLL

Each year the Director of LLL gives a State of the Laboratory talk, reporting to the staff some of the accomplishments of the past year, the present directions the Laboratory is taking, the new budget, and possible future trends. This article is derived from Roger Batzel's talk on April 6, 1976. Because the prime programmatic responsibility of the Laboratory is nuclear weapons research and development, representing 55% of our budget, a large portion of Dr. Batzel's talk dealt with the accomplishments and plans of this program. Some of these remarks, because of their classified nature, cannot be reported here.

Several years ago, when about 75% of the Laboratory's effort was in the nuclear weapons area, we made a policy decision to further diversify our programs, especially in the energy and environmental areas. We felt, in the long term, the Laboratory would be more stable and we would be in a better position to execute the weapons program, if we had a broader base of support. We looked hard at those areas where increased effort might complement and strengthen the weapons effort as well as make a broader and more valuable contribution to the country.

We organized major programs in laser fusion and laser isotope separation. We mounted projects based on Livermore-generated concepts of *in situ* coal gasification and the production of oil from oil shale. We initiated a geothermal power generation program, along with several smaller energy projects — such as solar ponds, wind energy, fly-wheel development, lithium-air-water batteries, and topping cycles. Some of these show promise of future expansion. We are starting a substantial effort in uranium hydrogeochemical surveying. Other promising energy concepts remain under study.

In the biomedical and environmental fields, we expanded our efforts along the lines of ERDA's wider responsibilities to understand the effects of all forms of pollution in the biosphere. In the biomedical area we are studying the possible correlation of these effects at the cellular level. In the environmental area, we are the lead laboratory in the nation's definitive study of the environmental impact of developing geothermal energy in the Salton Sea area.

Synergistic diversification has also been achieved through reimbursable projects, financed by other government agencies that need the expertise we can provide. For example, we have undertaken an important ordnance problem for the Air Force: the design of a modest-sized munition that can penetrate and destroy a thick, reinforced-concrete bunker or the heavy pilings of a bridge (Fig. 1).

A particularly important development in the reimbursable area during the past year has been our evolving relationship with the Nuclear Regulatory Commission. NRC is faced with abundant problems in regulating the nuclear industry. We are undertaking a number of projects for them, representing altogether a multimillion dollar effort, in areas such as facility safeguards and reactor safety. We expect this effort will continue to grow and that the relationship will be stable for some time to come.

In summary, I think our diversification policy has indeed made the capabilities of the Laboratory more widely available and of broader value to the country, while at the same time strengthening our ability to execute the weapons program. One example of this has been the just-completed accelerated test program, necessitated by the decision of the US and the USSR to sign a Threshold Test Ban Treaty. This Treaty limits the yield of each weapons test, and of each Plowshare explosive, to 150 kilotons. Within a limited amount of time, we were able to verify the feasibility of a number of high-yield designs, which will provide the nation with the flexibility to choose among several options for future strategic systems.

The accelerated program also helped us in providing a new strategic bomb, the B77, for the B-1 bomber and other strategic aircraft. The B77 can be released from a bomber flying at supersonic speed at a 100-foot altitude, a delivery mode that greatly increases aircraft survivability. We also developed an insensitive high explosive for use in the B77 to enhance safety in case of an accident.

[For other Laboratory accomplishments singled out by Dr. Batzel, see the box on the following two pages. His talk continues on p. 5.]

25 ACHIEVEMENTS DURING THE PAST YEAR

[In his State of the Laboratory talk, Dr. Batzel reviewed a number of LLL achievements. The list below reflects both the range of programs and work at LLL and some of our most important recent accomplishments.]

Weapons

- We conducted a successful series of high-yield tests that ended in March 1976, in anticipation of the effective date of the treaty limiting future underground test yields to 150 kilotons. As a result, the Laboratory has provided the nation with a series of options for future strategic systems.
- LLL is presently designing two new weapon systems, one strategic and one tactical, for the stockpile.

Magnetic Confinement Fusion

- On the 2XHB facility, we demonstrated a principle crucial to the mirror program: that plasma confinement time increases with plasma temperature. We also observed the highest plasma temperatures ever in a major fusion experiment, more than 250 million degrees F. This research, coupled with recent high-density experiments in the 2XHB, strengthens our belief that we can demonstrate the scientific feasibility of the mirror approach to fusion in the early 1980's. The MX experiment now under design is intended to do this.

Laser Fusion

- We passed a major milestone when we obtained the first direct experimental evidence that thermonuclear neutrons are produced in laser-driven implosions.
- Advances in solid state laser materials and other improvements promise a four to eightfold increase in laser power per dollar invested.
- We continued our systematic step-up in target irradiation facilities. The 0.5-TW Janus laser was operating; the 1-TW Cyclops came on line; assembly of the 2-TW Argus began; and the procurement cycle was started for the 25-TW Shiva system, to be housed in the nearly complete high energy laser facility.

Laser Isotope Separation

- We demonstrated the first macroscopic laser enrichment of uranium. Using our exceptional capability in detailed high-resolution laser spectroscopy, we demonstrated several other uranium enrichment techniques with ionization sources such as infrared lasers and electric fields.

Energy Programs

- In our *in situ* coal gasification program, a small underground coal fracturing experiment was carried out in Wyoming in preparation for a preliminary gasification experiment next Fall.
- A large, carefully instrumented laboratory retort for extracting oil from shale rubble was built and installed in support of the *in situ* oil shale program. The retort underwent a shakedown run and has been suitably modified.
- In the geothermal program, the first stages of our test facility began operation here at Livermore while we were conducting brine and materials studies both in California's Imperial Valley and here. We have designed a prototype turbine for harnessing the total flow of brine liquid and vapor, which will be tested shortly. We were asked to provide technical assistance to industry in developing geothermal power, and we are now working with San Diego Gas & Electric Company to this end.

• Our shallow pond approach to harnessing solar energy for industrial process heat continues to look very attractive. Testing of full-size ponds in New Mexico is almost complete, and the basic economic feasibility of the approach is being assessed with the help of an engineering firm. Our industrial partner, Sohio Petroleum, is now considering the installation of a large-scale solar heating system.

Biomedical and Environmental Programs

• Our discovery that high-speed, laser-based flow systems could be applied effectively to chromosome analysis and sorting has created a powerful new tool in cytogenetics.

• A complementary image-analysis system for chromosome measurement, CYDAC, reached the point of initial clinical applications—a prenatal diagnosis for a possible birth defect and quantitative confirmation of a microscopic chromosome defect in leukemia.

• A research project established the extreme sensitivity of mouse germ cells to radiation damage caused by low levels of tritium; the effort is now being extended to similar studies of the effects of energy-related environmental chemicals.

• A substantial study was initiated using mammalian cell systems to develop new methods of identifying environmental chemicals that are mutagenic or carcinogenic.

• In our new role as lead laboratory for a two-year study of the environmental aspects of geothermal energy, we have begun baseline measurements in California's Imperial Valley geothermal fields. This study will involve some 40 LLL investigators as well as many university contractors and several state and local government agencies.

• The ARAC (Atmospheric Release Advisory Capability) computer network, based at Livermore and connected to other ERDA laboratories, was established. It will make real-time predictions of the movement of toxic materials through the atmosphere in the event of such a release.

General Research and Development

• We synthesized solid (cryogenic) deuterium-tritium and made the first measurements of the mixture's electrical resistivity. This work has bearing on electrical methods of directing fusion targets to a precise position for laser irradiation.

• Our physicists developed the ability to measure fission cross-sections in the laboratory from unprecedentedly small samples, as demonstrated recently in an experiment with three micrograms of curium-245.

• In collaboration with LASL, we discovered and synthesized fermium-259, the most neutron-rich isotope ever made, and studied its spontaneous fission properties.

• We developed two new electromagnetic techniques for geologic probing. These have potential application in searches for underground coal seams, voids, and ore bodies and in diagnosing *in situ* resource recovery processes.

• Our engineers designed and implemented new large-scale integrated circuits (LSI) for weapons security applications as well as a new generation of downhole digital diagnostic systems that are cheaper, more reliable, and more versatile than previous systems.

• We perfected experimental techniques for the precise measurement of equation of state at extreme conditions—10 000 K and 1 GPa (10 kilobars) of pressure.

Reimbursable Programs

• For the Air Force we developed a Hard Structure Munition, a two-step HE system designed to first penetrate and then destroy reinforced structures.

• Under National Institutes of Health and ERDA sponsorship, we collaborated with the University of California at San Francisco in developing an automated sample changer that creates a significantly improved diagnostic tool for physicians needing trace element analyses of blood.

Fig. 1. The hard structure munition (HSM) was successfully tested last August against a reinforced-concrete block 9 m high, 9 m wide, and 4.5 m thick weighing 900 tonnes (top). The munition (middle) uses a "two-stage design with a shaped charge softening the target and allowing the main warhead to penetrate deeply. Detonation obliterated the target center (bottom) and tore apart the remaining concrete.

Where We Are

The fruitfulness of our diversification efforts and the achievements of the past year show that the Laboratory is in a very creative and productive period. I think several factors are involved:

- The Laboratory is staffed with very able people.
- Our organization and broad technical base stimulate innovation and achievement.
- Our country is now facing many problems and needs that are particularly amenable to solution through the applied technology skills in which the Laboratory excels.

Ernest Lawrence used to say that good morale is not a goal to be pursued in itself; it comes from doing good work. Good work starts with having very capable people; we strive to bring into the Laboratory people of the highest quality in all fields. I think we have succeeded in this goal; the best evidence of this is our record of achievement.

One reason we are able to attract and keep good people on the staff is that we maintain an environment of achievement. Two important elements of this environment are our broad technical base and our flexible organizational structure. At Livermore it is possible to find experts, research facilities, and engineering and fabrication resources in most fields of science and technology. This multidiscipline technical base was built primarily for the weapons program; it allows people to get things done in a way that often cannot be matched elsewhere. Scientists and engineers are attracted to this rich technical environment, and excellent technicians and craftsmen like to work as members of productive technical teams. This broad technical base is the main reason for our success.

We inherited our flexible organizational structure from Ernest Lawrence. It is built on a matrix of exciting programs supported by a core staff. We try to avoid rigidity while maintaining a reasonable degree of administrative effectiveness. This structure permits us to add new projects or change directions quickly when new ideas gain support. The value of this organizational structure has been recognized in a number of ways. Other laboratories have emulated our system; ERDA management has pointed to LLL as a model for other institutions.

Incidentally, our technical base, our ability to move quickly, and our reputation for problem solving have motivated government agencies and industry to request

our help in solving problems. The totality of these requests has been more than we can handle; priorities must determine what we do.

The third reason for our current success and productivity relates to the technological problems that face the United States. For many years basic science enjoyed an ascendant position in the country. Several years ago, for complicated reasons, especially the perception of serious national technological problems, a shift in priorities began. I think it is clear that we have entered a period of applied technology, where problem solving is the order of the day.

This Laboratory is well designed and staffed for doing applied science and technology. It was established a quarter of a century ago to work on high-priority programs involving complex science and technology. Problem solving has always been our main charter. We can do applied technology that simply cannot be done elsewhere — a big reason why we are prospering today.

We also do basic research related to our long-term programmatic goals. We are acutely conscious of the need for basic research to provide new data and ideas for our programs. And we have been working to maintain a reasonable level of such work. It's not easy to persuade sponsors who want *urgent problems* solved to see the need for basic research. The universities, which have a special charter in basic research, have had significant setbacks in recent years.

I am concerned about the national trend toward reduced basic research, although I am heartened by some proposed increases for the universities in FY 1977. In view of this trend, I asked for a survey of basic research at LLL. The finding was that the percent of the budget devoted to basic research, integrated across all Laboratory programs, is approximately the same today as it was five years ago — a level of about nine percent. The only difference is that it has now shifted to a much wider support base: each major program supports its own requirements for basic research. It is clear that we have been able to hold our own partly because basic research is fundamental to the development of advanced technologies. To do these programs, we must do basic research.

As I think everyone knows, our relationships at the Washington level have changed. Until a little more than a year ago we operated under the Atomic Energy Commission, whose charter was limited to the nuclear field. Our new sponsor, ERDA, is charged with the much broader responsibility of addressing the whole

Fig. 2. In current dollars (not adjusted for inflation), the Laboratory operating budget has increased about 80% for the five-year period shown here. The weapons budget has increased about 40%, and the aggregate budget of all nonweapons work has increased about 200%.

spectrum of energy-related problems of the U.S. We think we have made headway in showing the ERDA leadership how we can help, but we are still in a period of learning and adjustment in communicating and dealing with them. Dr. David Saxon, the new President of the University of California, has been very helpful to us in this period.

In fact, our whole network of communications has become more complex. In ERDA, we need to work with the Administrator, the Deputy Administrator, the six Assistant Administrators who are the Program Administrators, and many others. In Congress, we now have many committees who oversee various parts of the ERDA and Laboratory programs; Dr. Seamans has noted that ERDA interacts with 55 Congressional committees and subcommittees. We are also working with many Congressmen and their staffs. In the executive branch, it is important to keep the Office of Management and Budget informed.

Our relations with industry have also expanded. Since it is industry that must apply new technologies, ERDA, at the urging of Congress, has developed commercialization and technology transfer programs; industry must become a part of the team early on. Our program people are developing working ties with a wide spectrum of industrial firms and consortia.

This complexity of relationships will require a lot more time on the part of everyone -- both the administrative and the programmatic sides of the Laboratory.

FY 1977 Budget

I think the proposed budget for FY 1977 (Fig. 2) reflects a recognition at the national level of both the Laboratory's achievements and the ability of our staff to help solve national problems. I would like to make several observations:

- The budget shows an 18% dollar increase for the weapons program. This reverses the trend of the last four budgets in which there was a slight increase in dollars but not enough to keep up with inflation, forcing the real level of effort downward. I believe the reversal is due to a widespread conviction in the nation that we have been falling behind in defense, and that we need to correct the situation.

- There is continued growth in the energy and environmental fields. I am pleased with this increase, but I believe funding is somewhat low for the exploration of new technology areas. I'm not sure this will change, however, until the public realizes how serious the energy problem is.

• An important budget-related matter is our proposed manpower level for FY 1977, which reflects a proposed increase of 400 to 500 people. In Fig. 3 you will notice that the trend has been upward since FY 1974. An unexpected cut in that year's budget forced a layoff in the Spring of 1973. Present indications are that the staff level will go above 6,000 people in the next fiscal year. Given the nation's technical problems and the Laboratory's special capabilities in applied science, I believe this growth trend is likely to continue, although the future rate is not clear.

The proposed Congressional FY 1977 funding for equipment offers us a brighter outlook than we have seen for several years. The budget calls for an outlay of almost \$21 million, nearly a 20% increase over FY 1976 and much better than the lean years which started in FY 1973. Even though some of the increase will go to inflation, there is a very real improvement.

On the other hand, the facilities picture is not as bright — the President's budget includes funding only for the National CTR Computer Center building and for an addition to our biomedical building. We have not been able to get funds to relieve the proliferation of trailers and continued housing in old barracks buildings. We are trying, on an urgent basis, to get a large core structure to house a variety of

administrative and support operations now occupying space in the programmatic areas.

Future Directions

What about the future of the Laboratory? What I've already said indicates my belief that the future is bright indeed.

The turnaround in weapons funding, if approved by Congress, will allow us some growth in this area and will give us leeway to explore the new concepts we have been proposing for a long time.

In the magnetic confinement fusion program, the pace of progress has quickened in a striking way. The LLL mirror approach, with its potential economic and small-size advantages, is a very strong contender for ultimate power generation. I expect we will get support for the MX facility to test scientific feasibility. I hope we can get funding to begin this \$50 to \$100 million project in FY 1978 (Fig. 4).

The laser programs — fusion and isotope separation — have very bright prospects. Work on SHIVA, which will be the world's most powerful laser system for laser fusion research (20 to 30 TW), is coming along well; it is scheduled to begin operation at the end of 1977 in its own new laboratory. Discoveries made last year offer the promise of

Fig. 3. Manpower is a good index to the level of effort in our various programs. Over the five-year period shown here, the weapons effort has decreased slightly; however, FY 1977 looks like the turnaround point. The aggregate of all other programs for this period has increased about 75%, and the Laboratory total has increased about 20%.

Fig. 4. In 2XIIIB (bottom), plasma confinement occurs in a vacuum chamber buried inside the magnet coils. Plasma guns inject a burst of hot plasma into the chamber; this burst is trapped, compressed, and heated by the fields of the fast-gate and compression magnets. Twelve neutral beams further heat and sustain the plasma. Titanium-coated surfaces in the end chambers absorb any plasma that escapes confinement. Length between the mirrors is 1.5 m. MX (top), our proposed next-generation experiment to test the scientific feasibility of the mirror approach to fusion, will be much larger. Over 12 m high, MX will provide a transition from present small mirror experiments to large steady-state devices.

Fig. 5. This sample (right), containing 3 mg of uranium enriched to 3% by two-step selective laser excitation, symbolizes several advances in laser isotope separation technology. We have demonstrated the scientific feasibility of two- and four-step uranium enrichment. The Argus laser system (bottom), now nearing completion, spans the capabilities of existing laser systems, such as Janus and Cyclops, and the larger and more powerful Shiva system, scheduled for operation in late 1977. Argus will be able to operate at over 2 TW using two beams, as pictured, or at 4 to 6 TW if upgraded to four beams with advanced materials and components. Argus will be capable of driving high-density implosion experiments not possible with smaller systems.

Fig. 6. An overall view of the shallow solar pond prototype facility at the Sohio uranium plant in New Mexico. Ponds of two different designs are being tested and compared for efficiency and reliability. Solar-radiation intensity is recorded by the instruments at the left.

Fig. 7. Mobile geothermal-brine test unit being installed in the Salton Sea geothermal area. The test unit will be used in a series of scale-control and materials-evaluation experiments as part of our effort to prove the feasibility of the total flow concept for utilizing hot, high-salinity brines. Our experimental results here will also find application to other conversion concepts and geothermal resource areas.

Fig. 8. New oil-shale retort for extracting oil from shale rubble. This carefully instrumented retort is 6 m high and holds 6 tonnes of oil shale. A computer-controlled heater system minimizes external heat losses, allowing simulation of *in situ* retorting conditions.

Fig. 9. One example of our biomedical research is the automation of cytodiagnostic procedures. Shown here are computer displays of a normal (left) and a precancerous (right) human cervical cell nucleus. The clumpiness (optical texture) in these microscopic pictures can be measured by computer to provide diagnostic information about the cells. Such texture analysis has many potential applications in biomedical image processing; this particular example is directly applicable to automating the routine Pap test for cancer of the cervix.

upgrading SHIVA in the 1981 time frame and of shortening the time needed to achieve laser fusion goals (Fig. 5).

In the new energy programs, we are off to a good start. We are building a base of knowledge and experience in some challenging areas. I think we are in an excellent position to expand as more funds flow to energy R & D (Figs. 6-8).

The biomedical and environmental program is making good progress in its goal of assuring that energy resources are made available with minimal negative consequences to human health and welfare. Examples from each branch of the program are shown in Figs. 9 and 10.

The reimbursable area will continue to offer opportunity to apply in a wider fashion our special skills to the nation's problems.

In conclusion, I would like to congratulate the staff on an outstanding performance during the past year. May this coming year be as productive.

• • •

Following Dr. Batzel's talk there was the traditional question period. One of these questions has been selected to end this article. The question was: please list and discuss the five areas, both internal and external, which are of most concern to you.

My first area of concern would be the long-term ERDA-DOD relationship as it relates to nuclear weapons. The issue is settled for now. [In a January 21 hearing before the Joint Committee on Atomic Energy, Dr. Seamans said, "The full detailed recommendations were submitted to the President with the full concurrence of the Department of Defense. Overall, it is our joint judgment that the responsibility should be retained by ERDA."]

The second area, also an external one, concerns the best way for the Laboratory to interact with the rather complex ERDA administrative structure. One basic issue here is the question of whether the Laboratory will continue to have the necessary flexibility to pretty much choose its own balance of programs and structure their details.

The third area is both external and internal: what is the optimum size for the Laboratory? or is there an optimum size? Some size limitation may well be externally imposed on us. As to our internal view - we don't yet have a consensus as to what the right answer is. I myself feel it's at least 6000.

The fourth area concerns the organizational structure and communication network of the Laboratory. Externally we need to be structured to interact with the ERDA organization (with its six programmatic Assistant Administrators, who are

Fig. 10. Our environmental studies emphasize field measurements. This portable backpack instrument was developed to measure the effects of air pollution on trees. The device is placed over leaves to measure photosynthesis and transpiration. Patterns of pollutant-induced injury have been detected and are of use in understanding energy-related environmental impacts and in screening for the extent of damage.

responsible for an even larger number of Divisional programs), with Congress and its many committees, and with the University. The issue of internal management style has more to do, in some ways, with the competence of management than with its structure. But we recognize that there is room for improvement, and we are going to work hard to bring it about. One aspect of communications was the creation this past year of an Associate Director for Human Resources and Laboratory Relations.

My final area of concern is the continuing one of programmatic direction — are there important areas the Laboratory ought to be exploring? what kind of balance in program variety should be maintained? which programs should be continued and which phased out? This last question is perhaps the hardest to be faced: unsuccessful projects are relatively easy to deal with; the difficulty comes from a project that has reached many of its goals, is well-run, and is still doing useful but relatively unexciting work — in short, an “average” project that is using the resources needed to start new and more important projects. Fortunately our flexible organizational structure helps us greatly in solving such resource reallocation problems. As to the balance and breadth of the Laboratory programs, my personal feeling is that the mix should be about 50/50 between weapons work and other work.

Reprints of this article are available from the Technical Information Department, Lawrence Livermore Laboratory, Livermore, California 94550.

ENVIRONMENT, HEALTH AND SAFETY

LLL BIOMEDICAL AND ENVIRONMENTAL RESEARCH PROGRAM OVERVIEW

The chief objective of LLL's biomedical and environmental research program is to enlarge mankind's understanding of the implications of energy-related chemical and radioactive effluents in the biosphere. The effluents are studied at their sources, during transport through the environment, and at impact on critical resources, important ecosystems, and man himself. We are pursuing several projects to acquire such knowledge in time to guide the development of energy technologies toward safe, reasonable, and – in the broadest sense – optimal choices.

When the biomedical program began at Livermore in 1963, it was designed to study the dose to man from radioactive releases into the environment. The primary concerns were fallout, underground weapons testing, and potential Plowshare nuclear-cratering applications. The program contributed significantly to measurement technology, field studies, action mechanisms, transport and uptake models, and, ultimately, to comprehensive assessments of actual and potential nuclear effects.

In the past five years, anticipating national policy and the formation of ERDA, our focus has shifted toward a wider purview of energy sources in general. The nuclear skills have proved readily translatable, providing an excellent base for examining the environmental problems associated with new and old energy resources. Our choice of studies has been guided partly by ongoing technological work at LLL: the chosen style is to work in parallel with technologists toward supplying clean energy with minimal adverse effects on human health and welfare.

Our work has two essentially equal components: biomedical and environmental research. Biomedical research is mainly cellular and mechanistic, dealing with the search for early manifestations of injury in humans and with a general understanding of the processes of mutagenesis, carcinogenesis, and reproductive effects. Environmental research is macroscopic and comprehensive; it measures the flow

of pollutants from the energy technologies through the various ecosystems until they reach an important organism or man himself. This is accomplished through field studies and an overview function involving analysis and assessment of all available information.

Our program draws heavily on LLL and University capabilities in physical sciences, engineering, atmospheric sciences, computer sciences, sociology, and economics to develop the advanced instrumentation, measurement techniques, and analytical scope needed to characterize biological systems ranging from single cells to the biosphere.

Environmental Research

This effort is directed toward improved understanding of the health, environmental, and socioeconomic consequences of energy extraction and consumption, waste management, and related activities. This research encompasses activities in three major areas – marine biology, terrestrial ecology, and atmospheric sciences – and involves both laboratory and field studies. Our work includes numerical simulation of transport processes, instrumentation development, integrated assessments of energy-related impacts, and analysis of energy-policy considerations.

With respect to nuclear energy, our studies emphasize plutonium and other transuranic elements, focusing on their transport and long-term environmental behavior in terrestrial and marine systems. Our analyses of atmospheric-terrestrial exchange, at the Nevada Test Site, have provided important insight into the pulmonary uptake of plutonium, perhaps its major pathway to man. Coastal and oceanic studies have explored the environmental implications of coastal and offshore power-plant siting. At the Humboldt Bay reactor, Eureka, California, we have evaluated the dose to man via the marine food chain by studying the source term, radionuclide dynamics in the bay, and the radioactivity in oysters, clams, crabs, and bottom fish.¹ Similarly, for the two Diablo Canyon nuclear reactors now under construction near Morro Bay, California, we are examining marine plants and animals and will be looking for a variety of ecological impacts, including dose to man, when the reactors come on line.

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Of two other studies relating to nuclear energy, one is the development of a national advisory capability – ARAC² – to deal with any accidental radioactive release to the atmosphere. The second involves the biogeochemical cycling of radionuclides and potential dose evaluations at Enewetak and Bikini atolls (sites of post-World War II nuclear tests). An interesting biological find has greatly aided our historical reconstruction of how the current environments at the atolls have evolved. As described in the article beginning on p. 16, sections of brain coral show the heavy elements that prevailed when each growth ring was laid down, thus serving as an excellent radiological chronometer of the marine environment. In related work at the Marshall Islands, we have studied the ground water, wildlife, soils, and plants in a continuing assessment of how and when the several hundred indigenous Marshallese can return to the islands.

In nonnuclear energy areas, similarly integrated approaches to source terms, pathways, and dosimetry are being applied to a wide spectrum of problems. Some of our activities are studies of effluents from coal-fired plants, investigation of the sources and effluent-related role of dissolved organic material in fresh water, and studies of the transfer of trace contaminants in food pathways. For example, in an attempt to learn more about the flux of trace elements through the biosphere into human food chains, we are surveying such elements in market milk. Samples are being collected from specific sites, as well as from a

broad national network. By correlating the results with source terms and fodder samples, we can explore the dynamics of transfer and evaluate the usefulness of market milk as an indicator of trace-element environmental pollution.

Activities in the nonnuclear area include such diverse concerns as the effects of offshore petroleum exploitation on benthic (ocean-floor) biology (see Fig. 11) and the creation of a group of coordinated, carefully validated numerical atmospheric models for application to local pollution control, regional transport, and global effects such as inadvertent weather modification. We are developing tools and understandings to diagnose pollutant impacts on plant ecosystems. We are also studying several problems in assessment and evaluation of the consequences of energy-related policies and activities.

Of major importance is our new role as the lead laboratory in the assessment of the health, environmental, and socioeconomic consequences of geothermal-resource development. These studies, announced last month,³ are discussed in more detail in an article beginning on p. 21. This project is ERDA's first prototype of a totally integrated environmental assessment of a developing energy technology.

Biomedical Research

The major emphasis in our biomedical research is on genetics, carcinogenesis, reproductive biology, and biophysical cytology – the technology and science of probing cells for their physical, chemical, functional, and morphological (shape, structure, and appearance) properties.

The genetic process, including its modification through mutagenesis, is the key to understanding the long-term biological significance of physical and chemical agents in the environment. The two major health hazards of low-level radiation exposure are both genetic: one in the classical sense of heritable mutations, and the other involving somatic (body cell) mutation at carcinogenesis. The same is likely to prove true for energy-related chemical effluents and for other industrial chemicals.

To deal effectively with the genetic challenge, we need profound knowledge of the genetic mechanism, of mutagenesis, and of the biological significance of mutation rate. Also required are tools to measure genetic integrity and to monitor – rapidly and sensitively – for mutations and mutagens. Our efforts in this direction include studies on chromosome structure and aberrations, conditional cellular mutants,

Fig. 11. Underwater view of a 15-cm-diam tar mound in the Santa Barbara channel (California) at a depth of about 17 m. Natural oil seepage near Santa Barbara is being used as a field laboratory to study the effects of oil pollution on benthic (ocean-floor) life. We are studying such benthic communities to establish a baseline before resumption of oil drilling. The small droplets in the background are responsible for most of the seepage; these eventually float to the surface in large numbers, forming an oil slick.

metabolic mutants, and new approaches to the screening of mutagens. We are studying the chemistry of DNA, the primary genetic material, and ways in which information is transferred from DNA to the rest of the organism. The remarkable DNA of the kangaroo rat has provided us a rich source of insight into the organization of DNA (see the article beginning on p. 26). The parallel effort in carcinogenesis includes development of a quantitative cytochemical approach to cancer diagnosis and new methods for screening carcinogens by *in vitro* (outside the living body) techniques, such as the scoring of transformed cells in cell cultures.

Reproductive biology concerns the uniquely sensitive, vital processes of gametogenesis (formation of eggs and sperm), fertilization, and embryogenesis (development of the embryo). These processes, which express and are the expression of a large part of genetic effect, can enormously amplify what otherwise would be trivial physiological or pharmacological perturbations in organisms. Their exquisite sensitivity is being exploited to develop assay methods for potential mutagens and teratogens (agents that produce death or deformity of embryos). Studies in this area include the separation of testicular components, the development of methods to measure altered sperm structures and functions,⁴ continuing investigation into the remarkable sensitivity of developing oocytes (eggs) to radiation and chemicals, and comprehensive studies on chromosomes at each stage of the reproductive process.

As part of our work with oocytes, we have discovered an extreme radiosensitivity of mouse eggs to low-level exposure from tritium and gamma irradiation. The relative biological effectiveness of tritium has been shown to increase with length of exposure. Tritium is an important nuclear byproduct that will be generated in increasing amounts in coming years; it has ready access as water to living systems and passes freely from mother to developing offspring. The special sensitivity of the mouse egg is limited to the period immediately after birth, giving us a unique test system for studying the all-important biology of low-dose and low-dose-rate effects. Parallel studies with chemical agents are now under way.

Our work in biophysical cytology encompasses measurements of the volume, shape, DNA content, motility, and radioactivity of cells or subcellular organelles such as chromosomes. This is a breakthrough area because of technological developments in automated image analysis and flow systems. These

technologies offer enormous capacity and a rapidly growing diversity of cellular probes. They promise a practical answer to sorely needed improvements in chromosome analysis and cancer diagnosis, as well as identification of somatic mutations as rare, unpredictable events occurring in single cells. LLL developments in this area include a computer-based image analysis system (CYDAC) for chromosomes and other cellular images,⁵ three-dimensional electron microscopy, development and diverse applications of flow systems,⁴ new approaches to measuring cell-cycle kinetics, and improved cytochemical methods for fluorescent and absorption cytophotometry.

CYDAC (for cytophotometric data converter) is an LLL-perfected microscope-computer hybrid for measuring DNA in chromosomes. This approach can detect a DNA loss as small as 5 fg (5×10^{-15} g). It combines a visible-light microscope, special scanning electronics, and automated image processing (see Fig. 12). In one CYDAC application, we have learned how to determine — on the basis of precise DNA measurements — which parent the chromosome came from. This capability creates a powerful new tool for studying the mechanism of genetic inheritance and the cause of mutations.

In flow systems, cells in suspension are carried single-file by laminar flow past an optical or physical

Fig. 12. CYDAC scanning cytophotometer operates at the limit of optical resolution, digitizing human chromosome preparations as seen through the microscope. The operator here is using the focus-assist device, which optimizes focus to 0.1 μ m. Pictorial data are transmitted directly to the computer, which reconstructs the image and displays it on the television monitor above CYDAC. Subsequent computer analysis estimates the amount of DNA associated with each chromosome, comparing it with the norm. We have been using the system recently for prenatal diagnosis and for leukemia studies.

sensor. Typical processing rates are 1000 cells/s; fluorescence detection, such as measurements of cellular DNA, can be accurate to 1 or 2%. Flow sorters have the added ability to sort the cells on the basis of measured properties. Perhaps the most exciting part of this new technology is our recent discovery that it can be used to measure and sort chromosomes at unprecedented rates and accuracies (see the article beginning on p. 31).

Summary

This brief overview provides some sense of the main themes of our biomedical and environmental research program. Four aspects are highlighted in the articles that follow. In reality, the program consists of some

50 ERDA projects and 15 others for the Environmental Protection Agency, the National Institutes of Health, and other agencies. Integration of the themes that overlap and weave through the projects is the glue that holds the program together, the rationale for why it belongs in a national laboratory, and the only hope for approaching our overall goal in the face of the complexities of living organisms and the intricacies of environmental influences.

Key Words: biomedical research; environmental research; nuclear energy - environmental and biomedical effects; nonnuclear energy - environmental and biomedical effects; genetics; carcinogenesis; reproductive biology; biophysical cytology; energy-related effluents.

CORAL AS A RECORDER OF RADIOISOTOPE CONCENTRATIONS

The post-World War II nuclear tests on Bikini and Eniwetok atolls left behind an environment from which we hope to learn more about the processes affecting radioisotope distribution and removal from the biosphere. A complete record of the isotopic concentrations and their variations with time, for all radioisotopes of interest, is basic to this study. Such records have now been obtained from specimens of living coral dredged from the lagoon bottoms. The lagoon-water concentrations of even the long-lived fallout components have declined a hundredfold or more since the tests. However, using the coral chronometer, unexpected past changes in radioisotope concentrations well beyond experimental uncertainties are now observable. Detailed studies into the retention and release mechanisms will be required for predicting such variations.

Our environmental studies, designed to trace the history of radioactivity changes at Bikini and Eniwetok since the nuclear tests of the 1950's, have been expedited by the discovery that brain corals - *Favites virens* (Fig. 13) - show evidence of the elements that prevailed when each growth ring was laid down. Sections of these corals serve as excellent radiological chronometers of the marine environment

over succeeding years. The record is now almost complete. We have found one brain coral, for example, that was in the Bikini lagoon before 1954 and was still living and growing in November 1972 when dredged up from a depth of 29 m at Sampling Station B-3 near Bravo Crater, the largest of the Bikini test series craters (see Fig. 14).

Only the outer surface of a coral is alive. The bulk is made up of tiny calcium carbonate skeletons of countless individual polyps cemented together. As the coral grows, each generation incorporates into the matrix a representative sample of the elements in its environment. Seasonal variations in the growth rate show up as differences in the density of the matrix. Thus it is possible to date particular events in the

Fig. 13. The large, rounded object is a brain coral at home on the floor of a Marshall lagoon.

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Fig. 14. Bikini Atoll showing Bravo Crater, sampling Station B-3, and isoactivity lines of americium-241 (in mCi/km^2) in the upper 2.5-cm layer of sediments in Bikini Lagoon ($1 \text{ mCi}/\text{km}^2 = 37 \text{ Bq}/\text{m}^2$).

coral's life in a manner similar to tree-ring dating. Other coral species also trap radionuclides in their skeletons, but none discovered so far has the interpretable yearly growth-band structure that makes brain coral unique.

Interpretive Scheme. To establish the interpretive scheme for brain corals, we carefully sliced a specimen into 2-cm slabs, cutting from the top toward the base to avoid spreading the radioactive contamination upward. We washed the slabs in water and dried them in an oven at 110°C for a week. Then we placed one slab in contact with no-screen x-ray film for 60 days. The resulting autoradiograph is reproduced in Fig. 15a, with the regions of highest density — corresponding to the highest radionuclide concentrations — outlined to make them more obvious. These bands represent activity incorporated into the coral during the nuclear tests of 1954, 1956, and 1958.

At the same time, we took x radiographs of an adjacent slab (Fig. 15b). Not all the growth-band boundaries are equally clear here; other exposures at different energies were needed for defining all the bands. Eventually it became possible to associate every part of the coral — at least in the central wedge — with the time when it was growing. The average annual-band width was found to be $8.1 \pm 2.2 \text{ mm}$.

The next step was to separate the different growth bands. For this we used a bandaw, first to remove the nonlinear and convoluted growth on the sides of the central wedge and then to cut apart the different bands. The saw cut took about 1 mm of each annual

band, unavoidably destroying that portion of the record. The rest of each band was ground into powder in a ball mill so that a weighed representative sample could be taken for analysis.

Analytic Methods. By methods described in more detail elsewhere,^{6,7} we analyzed each powdered sample for gamma-emitting radionuclides, using low-background, Compton-suppressed $\text{Ge}(\text{Li})$ solid-state detectors and pulse-height analyzers. Figure 16 is a gamma-ray spectrum from a 12-g sample of the 1954-55 growth section (the only section not distinguished by individual year). Each prominent gamma-ray peak is identified in this figure. The unidentified peaks represent naturally occurring radionuclides and detector background.

The samples were then analyzed radiochemically for strontium-90, polonium-210 (lead-210), and plutonium isotopes, using low-background beta detectors and alpha spectrometry. The plutonium isotopes were further separated and individually measured by mass spectrometry. The extremely low background of the alpha detectors permit plutonium-238 and plutonium-239/plutonium-240 measurements at concentration levels of 0.2 mBq/g .

Results. It was believed at one time that radionuclides such as plutonium, once buried in sediments, were to all intents and purposes gone forever. We now know that this is not true. Lingering traces of plutonium in even the most recent coral growth bands at Bikini and Enewetak are clear evidence of radionuclides returning by unknown

(a)

(b)

Fig. 15. (a) Autoradiograph of a coral slab, superimposed on a photograph, verifies the presence of bands of radioactive contamination. The bands have been enhanced for better visibility. (b) X radiograph shows the annual growth bands that make brain coral uniquely suited for recording radioactive contamination. Additional exposures at different x-ray energies are needed for complete definition of band boundaries. Dashed lines indicate the section used for radiochemical analysis.

Fig. 16. Gamma-ray spectrum of a 12-g sample of the 1954-55 growth section of a Bikini brain coral. The identified photopeaks are those for radionuclides associated with nuclear debris. The unidentified photopeaks are those from background and naturally occurring radionuclides.

mechanisms from the sediment reservoirs into the lagoon water.

Our analytic results can be divided into two areas of interest: those relating to radionuclide concentrations and isotopic ratios, and those relating to physical processes (exchange and release rates) in the lagoon. The most important result of the former is unexpected and unpredictable fluctuations in the isotopic concentrations. Figure 17 shows, for example, how the plutonium-239 concentrations at Bikini have declined with time and also how the plutonium-238, plutonium-240, and plutonium-241 concentrations have varied with respect to plutonium-239. The sharp initial decline in the plutonium-239 concentration is expected; the North Equatorial Current sweeps continuously through the eastern end of the Bikini lagoon, quickly removing the plutonium from the lagoon water. The much slower, subsequent decline denotes leaching from the atoll's soils and sediments.

The sudden changes in concentration and especially in the isotopic ratios of plutonium are hard to explain in detail, although the general picture is clear. These changes are evidence of the nonuniform distribution of plutonium isotopes at Bikini. The different nuclear events deposited locally different isotopic ratios of plutonium. Even with a constant release rate, shifting currents would expose specific corals to varying isotopic ratios. Hence there appears no way, short of complete knowledge of each isotopic distribution and variable lagoon-bottom currents, to predict unequivocally the behavior of all plutonium isotopes from assessment of a single isotope.

Similar erratic changes in plutonium-isotope ratios have been measured in Enewetak brain coral.

Despite the uncertainties, we can use a simplified model of the lagoon environment to describe the

Fig. 17. Variation with time of the plutonium-239 concentration and plutonium-isotope-activity ratios at Station B-3 in Bikini Lagoon, based on analysis of coral growth bands. The variations in isotopic ratios are not due to different responses of the growing coral; they indicate environmental shifts, such as changing current patterns, that exposed the coral to contamination from different sources.

exchange and recycling rates of specific radionuclides. Figure 18 is a simplified input-output diagram of a contaminated lagoon and its surroundings. In this diagram, k_1 is the exchange rate constant (in yr^{-1}): the rate at which a radioisotope of interest enters the lagoon from the ocean. The recycling (release) rate constant k_2 , also in yr^{-1} , is the rate at which the radioisotope enters the lagoon from the atoll's soils and sediments. The n values are quantities of the radioisotope: the amount entering the lagoon from the ocean (n_1), present in the lagoon water (n_2), and entering the lagoon from atoll sources (n_3).

None of these rates or amounts is directly measurable, but they may all be derived from analysis of the coral growth-band data. A semilogarithmic plot of the concentration of any particular radionuclide shows a steep decline that levels out, after about five years, to a much smaller decline (Fig. 17). The slope of this later segment is proportional to k_2 , the recycling rate. Table 1 gives k_2 values derived in this way for various radionuclides.

Extrapolating the k_2 slope back toward the start of the graph, we can subtract from each year's data the contribution due to leaching from sediments. The balance, when replotted on the same graph, represents the decline in concentration that would have taken place in the absence of leaching. The slope of this line is proportional to k_1 , the exchange constant. Since k_1 is independent of the particular isotopic species, all values of k_1 can be averaged to obtain a value of $1.98 \pm 0.14 \text{ yr}^{-1}$.

Table 1. Release rate constant k_2 for specific radionuclides detected in a brain coral specimen from Bikini Lagoon

Radionuclide	k_2 value
^{239}Pu	0.07
^{240}Pu	0.07
^{241}Pu	0.06
^{238}Pu	0.13
^{155}Eu	0.06
^{207}Bi	0.13
^{60}Co	0.12

Fig. 18. Schematic input/output diagram of a coral lagoon. The constants k_1 and k_2 are the rates at which a radioisotope of interest enters the lagoon from the ocean and from atoll sources, respectively. For any given isotope, n_1 is the amount entering from the ocean, n_2 the amount in the lagoon, and n_3 the amount entering from atoll sources.

This mean k_1 value, implying that the lagoon water is replaced every six months, disagrees strongly with previous estimates based on physical circulation data alone.⁸ Those estimates predict about seven changes a year. Evidently there are mechanisms in the atoll environment, quite apart from leaching and re-solution, that slow the washout rate. This discrepancy should be borne in mind by anyone trying to assess the availability of pollutants to marine organisms in such environments.

The other surprising result from Table 1 is the release rate for plutonium-238 — twice that of any other plutonium isotope. There is no known biogeochemical mechanism that can fractionate plutonium isotopes to produce such a discrepancy. The only reasonable explanation is that the excess is entering the lagoon from a ^{238}Pu -rich region or source that is exposed to a much-faster-than-normal release mechanism. This is further evidence of the heterogeneity of the lagoon environment.

Key Words: environmental research; Bikini Atoll; Enewetak Atoll; environmental contamination; coral; radioactive fallout; plutonium isotopes; isotopic ratios.

ERDA has designated LLL to head up a two-year environmental study that should allow government, power-company officials, and other interested parties to assess the impact of geothermal development on California's Imperial Valley. The study, funded at \$2.4 million for FY 1977, will involve some 40 LLL investigators, 25 university contractors, and several state and local governmental agencies. Work on the project is now under way.

Major research areas of this coordinated study include air and water quality, effects on the ecosystem, subsidence and induced seismicity, health effects, and socioeconomic impacts. The study will include an integrated assessment to facilitate decision-making.

California's Imperial Valley is traversed by one of the nation's most vigorous and potentially productive high-temperature geothermal regions, the Salton Trough, which extends northward from the Gulf of California and includes the Salton Sea and environs (see Fig. 19). According to present estimates, the recoverable electrical energy from this trough is between 80 000 and 200 000 MW-yr; over half of this stored energy resides in the high-salinity brines of the Salton Sea geothermal area. This Laboratory is developing a process (the total-flow process) for converting the thermal energy of these brines to electrical energy⁹ and has now commenced field experiments to prove the feasibility of the process.¹⁰

In addition to its geothermal resources, the Imperial Valley contains other major resources with which geothermal-energy development must exist in harmony. The most important is an extensive agricultural system, which in 1974 produced crops and livestock valued at \$550 million. Another resource is the Salton Sea, California's largest and most productive inland fishery, which with its neighboring wildlife refuges is an important wintering area for large flocks of migratory birds traveling the Pacific flyway. Both of these resources depend on an adequate volume of fresh water imported from the Colorado River. Salt accumulation is a problem in both systems, and a sizeable investment has been made in irrigation drainage designed to control salinity in the agricultural soil.

The driving force to undertake large-scale environmental studies in the Imperial Valley at this

time is ERDA's cost-sharing agreement with the San Diego Gas & Electric Company (SDG&E) to build and use a 10-MWe pilot plant near Niland, California. This test facility (Fig. 20) is now in operation. If successful, it will be followed by larger facilities. Other public and private organizations are actively exploring the Imperial Valley resources, and it seems very likely that the valley will soon become a major site of geothermal development. The Mexican government has already built and is operating a 75-MWe power plant at Cerro Prieto, about 32 km south of the border.

ERDA, through its Division of Biomedical and Environmental Research, has designated LLL as lead laboratory for carrying out a long-term project to acquire complete understanding of the valley's environmental quality before any major development. In this task, LLL will coordinate its own efforts with those of some 25 university contractors (including those funded by other federal agencies such as the National Science Foundation), plus several state and local governmental agencies. The funding for FY 1977 is \$2.4 million. The project's purpose is to ensure that development proceeds on an environmentally sound basis. The principal research areas are air and water quality, effects on the ecosystem, subsidence and induced seismicity, health effects, and socioeconomic effects. A strong thrust is to integrate the output of these studies into a single overall assessment that includes not only data from the LLL research but also that from other identified programs.

Air Quality

Nearly all the proposed methods for harnessing geothermal energy result in the collection and venting of noncondensable gases composed mainly of carbon dioxide with lesser amounts of hydrogen sulfide, ammonia, and methane and trace amounts of other gases including radon-222. Volatile compounds of arsenic, mercury, and other elements may also be present in the noncondensable gas fraction. An important part of the air-quality program will be qualitative and quantitative analyses of these gases to provide a source term for future assessments. Other potential sources of airborne effluents, such as cooling towers, spray ponds, and brine sumps, will be evaluated.

Baseline and continuing measurements will be made by six fixed-location sampling stations. Each station

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Fig. 19. Imperial County, California, is traversed by the Salton Trough, which is roughly bounded by two major faults and includes the heavily agriculturalized Imperial Valley. False-color satellite view (below), in which red represents green foliage, shows extensive agricultural development. Geothermal areas now being considered for major development lie within the valley or immediately to the east.

Fig. 20. Aerial view of the ERDA/San Diego Gas & Electric Company 10-MWe pilot plant now in initial phases of operation near Niland, California. This plant ultimately will be supplied by two wells producing brines with more than 30% total dissolved solids. Spent brines will be injected back into the ground through a third well. One function of this facility is to permit studies of the long-term environmental impacts of large-scale geothermal development in the Imperial Valley.

will have sensors to measure windspeed and direction, temperature, relative humidity, and the concentrations of several pollutants including hydrogen sulfide, sulfur dioxide, ozone, carbon dioxide, and ammonia. Particulates and rain will be collected for further chemical analysis, as well as for basic data on mass loading and rainfall. A mobile field laboratory will be equipped to make not only all the above measurements but also those of nitrogen oxides, hydrocarbons, hydrogen fluoride, peroxyacetyl nitrates (PAN), and radon daughters; visibility, noise levels, and odor will also be monitored. The primary purpose of the mobile laboratory will be to make source-related measurements, supplementing the overall measurement capability. These fixed and mobile facilities are more fully described elsewhere.¹¹

A secondary goal of the measurement program will be to distinguish between pollutant concentrations arising from the effluents of geothermal-energy recovery and those arising from other sources (agricultural and industrial activities, importation from other areas). Some use of aircraft, for area-wide measurements of specific pollutants, is contemplated for this purpose.

One of the anticipated problems is sulfur emissions, primarily hydrogen sulfide. H_2S has been a nuisance to humans at other geothermal-development sites; it also undergoes atmospheric conversion to SO_2 and SO_4 . (The conversion rates, which are not well known, probably depend on the presence of other compounds such as oxidants and particulates. Additional laboratory studies will be performed as necessary to determine these rates.) Several sulfur compounds, particularly sulfur dioxide, are known to affect plant

growth. The association of sulfates and particulates has also been implicated in reduced visibility and some health effects.

Existing advanced models of atmospheric transport and diffusion¹² will be adapted to provide an air-quality model for the Imperial Valley that will include the chemical transformations of sulfur compounds. One goal will be to predict the concentration of geothermally derived pollutants for several developmental scenarios. This, coupled with data on the response of plants, animals, and humans to various pollutant levels, will be an important input to cost-benefit evaluations of various effluent-control options.

Water Quality

Utilization of the valley's geothermal resources will result in large volumes of spent brines whose salt content, in some cases, is greater than 30% dissolved solids by weight. These brines are a potential threat to the soil quality of adjacent agricultural land and to the overall water quality of the Salton Sea. The problem is well recognized. Present plans call for injecting the spent brines into the ground deep enough to prevent communication with shallow aquifers. Ponds and brine sumps may be required, however, as temporary holding areas.

Because the valley's water quality is already a matter of great concern, we are projecting a fairly extensive series of measurements to assess the present water quality and monitor the possible effects of geothermal development. We will determine the elemental content of processed brines and make many measurements of irrigation and drainage water both

upstream and downstream of developmental sites. A goal is to detect quickly any accidental communication between processed or reinjected brines and existing water-flow channels.

Another important part of the overall water-quality program is to assemble all available data on the valley's total water balance, including identification of significant sources and sinks and the regional and local flow rates. Some additional hydrologic investigations are anticipated in the vicinity of each developmental site to determine shallow groundwater environments and possible relationships between the shallow aquifers and the geothermal reservoirs.

Effects on the Ecosystem

The major ecosystems of concern are the agricultural lands, the Salton Sea, and the area's wildlife refuges. A primary goal is to develop enough information to predict the potential long-term effects of geothermal development and to institute remedial measures if necessary. For example, one easily identifiable concern is the potential impact of airborne effluents on agricultural productivity. Such effects will be studied by laboratory and field fumigation experiments to develop impact models (see Figs. 21 and 22). We intend to study the effects of brine-sump seepage, cooling-tower drift, and well blowouts near the developmental sites, to ascertain the results should these occur.

Aerial surveys will augment the ecological studies. Multispectral aerial photography will be used to assess vegetative stress, particularly near developmental sites. This technique plus infrared spectrometry will be used to investigate other environmental impacts such as brine seepage. Airborne radiological surveys will be

used to determine any changes in the radiation environment because of geothermal development.

Baseline and continuing measurements will establish the species composition of aquatic and terrestrial ecosystems. We plan to measure the concentrations and variability of trace elements in agricultural soils and crops, domestic animals and milk, and indicator wildlife species, with emphasis on the trace elements present in geothermal fluids. We intend also to measure the salinity of agricultural soils throughout the valley.

Because the ERDA-SDG&E pilot plant is located near a waterfowl refuge (Fig. 23), particular attention will be focused on assessing the impact on this wildlife community. Two sources of concern are airborne effluents and noise. We plan to investigate both.

Subsidence and Induced Seismicity

As shown in Fig. 19, the Imperial Valley has a significant earthquake potential. Measurements also indicate that the valley as a whole is undergoing right-lateral horizontal movement, and that its center is subsiding relative to its walls. Because both subsidence and induced seismicity are anticipated problems that might accompany geothermal development in this region, an extensive program is planned to assess their potential effects.

Subsidence has been found at other sites of hydrothermal development. We hope that the reinjection of spent brines will be an effective control in the Imperial Valley. But the potential effects on the valley's gravity-flow irrigation and drainage systems and the water boundary of the Salton Sea are serious enough to justify a significant research and monitoring program. Plans call for extending an existing interagency subsidence-monitoring network to provide

Fig. 21. Field fumigation experiments at the Agricultural Research Service Field Station near Brawley, California. Plants are briefly exposed to a gas mixture simulating the noncondensable gases to be released by geothermal power plants. Transpiration and photosynthesis rates are measured during the exposure period.

Fig. 22. L.L. ecologist measures transpiration and photosynthesis using a leaf-diffusion parameter. Transpiration (water release) is measured by recording the rate of humidity change in a small chamber. Photosynthesis is measured by exposing the leaf to ^{14}C -labeled carbon dioxide, excising the exposed leaf, and later measuring its carbon-14 content.

more detailed coverage where geothermal development seems imminent. Existing data will also be collected and interpreted.

Reinjection of fluid into wells has been observed to induce seismic activity in other areas of the U.S. Although the low-pressure injection planned for the Imperial Valley has not been implicated in causing seismic activity, we consider it necessary to monitor present activity near potential developmental sites to gain baseline data on natural rates, intensities, etc., prior to development. Several new, permanent vertical seismometers will be installed to supplement the seismograph network now maintained in the valley by the U.S. Geological Survey. Studies will also be made to determine whether additional instruments, such as tiltmeters and water-pressure gages, are needed.

Another network is planned for monitoring horizontal displacements near the ERDA-SDG&E pilot plant. Again, this network will supplement an existing USGS net whose spacing is presently too large to be useful in determining small, local distortions.

A modeling program may be undertaken to understand geothermal-reservoir behavior as it relates to subsidence. These studies will be based on the properties of reservoir rock, mathematical and physical flow models, equilibrium analysis of geologic structure, and other relevant data. We intend to review available information for the purpose of assessing the expected environmental effects should subsidence occur.

Health Effects

The major pollutants from geothermal development are well known, and their potential health effects have been studied for other reasons. Our first goal is to define any needed additional fundamental research. As data become available from effluent characterization measurements, other pollutants might emerge that would require specific work.

The predicted pollutant concentrations from atmospheric modeling will be examined closely for assessment purposes, especially the predicted concentrations of sulfate aerosols and hydrogen sulfide. Some research may be desirable on variations in the human detection threshold for hydrogen sulfide and the psychological effects of its odor. Work may be desirable, also, on the basic health state in the Imperial Valley, particularly as regards morbidity from diseases believed to be related to air pollution.

Socioeconomic Impacts

We have projected a long-term study of the valley to characterize its socioeconomic system and trace alternative developmental scenarios to their

Fig. 23. One of the wildlife refuges bordering the Salton Sea. About 100 000 migratory ducks and geese per day use these refuges in season. This spot is about 1.5 km north of the pilot plant shown in Fig. 20. The effects on this bird population of building and operating geothermal power plants are being studied.

conclusions: e.g., sectors likely to be positively and negatively influenced, multiple-use potentials for geothermal resources and energy, redistribution effects within the valley, socioeconomic resources to deal with change, and possible agreements (or disagreements) among governmental agencies about optimum rates of geothermal development. Studies of the direct socioeconomic impacts are being supported mainly by the National Science Foundation. We are concentrating on the indirect effects and depend heavily on data generated by the other programs within the overall project. The emphasis will be the fiscal and social impacts of geothermal development on the valley: on employment (including alternative employment), land conversion, population shifts, water-management programs, agricultural crop and irrigation substitution trends, county growth patterns, and the like.

Integrated Assessment

Our integrated assessment program will have several functions including central responsibility for data accumulation and management (internal and external programs) and for coordination of all modeling activities.

We will examine several broad, regional issues such as the legal and regulatory aspects of geothermal development, recommending changes as appropriate. High priority will be given to assessing the valley's water resources and the competing demands expected to be placed on them. It is important to delineate such problems carefully in advance, while there is adequate time to solve them; for example, to study several options for the needed heat-sinks, such as dry cooling towers.

Another important function will be to integrate all available information on emission-control options and to develop estimates of cost vs benefit for each option.

Finally, the integrated assessment program will be responsible for providing effective information transfer to regulatory, legislative, planning, and developmental groups with decision-making authority to implement the project's recommendations. Ultimately, the success of this function will determine the success of the project.

Key Words: environmental research; environmental studies - Salton Sea, Calif.; geothermal energy - environmental studies; Salton Sea geothermal area; environmental impact; environmental studies.

ENERGY TECHNOLOGY AND THE GENOME

In pursuing our goal of understanding the effects of energy-related effluents on man and the environment, we are studying mutagenesis: injury to the genome. Our work in mutagenesis has involved basic research into the organization of the genome — the cell's genetic material — and its major constituent DNA. For several years, we have studied intensively the remarkable DNA of the kangaroo rat. These studies have provided a rich source of insight into how DNA is organized, giving us a better understanding of how mutagens act in man and other organisms.

Mutagenic injury, which may be caused by energy-related effluents (see box on facing page), results from physical or chemical damage to the genetic material (DNA and RNA) in the cell nucleus. We have been studying the cell's genetic system because a detailed knowledge of it is needed to understand mutagenesis. The genetic system is the cell's information-processing system and is involved in all

vital activities such as growth, reproduction, regulation, and repair. This system includes: (1) the genome for information storage and its controlled release, (2) the RNA interpreting subsystem, and (3) the protein-synthesizing subsystem leading to many of the ultimate structural and functional molecules of the body (structural proteins, enzyme catalysts, transport proteins, hormones, and the feedback controls to the information storage system). Our studies have been concerned with the genome, the set of chromosomes of the cell carrying the complete set of genes for a particular organism (Fig. 24).

Chemical or radiation injury to the genetic system leads, through bond breakage or more complex chemical reactions, to the production of defective information in the genome. If this injury occurs in the egg or sperm, the defective information may be expressed as inherited birth defects in later generations: for example, sickle cell anemia (defective transport protein), cleft palate (defective structural elements), or mental retardation (defective enzymes of amino acid, carbohydrate or lipid metabolism). If the

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ENERGY TECHNOLOGY AND MUTAGENESIS

Mutagenesis in its broadest sense -- including somatic and germinal effects, point mutagenesis, chromosome breakage (clastogenesis), and carcinogenesis -- is currently the dose-limiting biological criterion for setting radiation standards. This same dose-limiting genetic criterion will probably apply to many other energy effluents and to the spectrum of commonly used drugs, food additives, and the chemicals of industry and agriculture. Understanding such effects and developing tools for their assay present a critical challenge to a technological society and are exciting areas for biological research.

A variety of pollutants arise from energy technologies, and these are, of course, a major concern to ERDA. Their potential implications for man's health and for his environment must be monitored. Fossil-fuel combustion produces many organic and inorganic pollutants: polycyclic hydrocarbons, some of which are known to be biologically converted to carcinogens and mutagens; oxides of nitrogen, which can at least potentially be converted to mutagens; oxides of sulfur, which can react *in vitro* like mutagens but are not known to do so *in vivo*; and heavy metals, like lead and platinum from transportation usage and mercury and thallium from coal, which could well be mutagens but whose potential is largely unexplored. Geothermal technology releases hydrogen sulfide, a toxic gas of unknown mutagenic potential, and a variety of trace metals that need further study. Genetically damaging trace-metal release and radiation can also arise from nuclear installations.

The major common pathway for mutagenic injury from these energy-related pollutants would be physical or chemical damage to nucleic acids in the cell nucleus. Such genetic damage can have several effects. It can so disrupt the cell's machinery that it is cell-lethal; it can grossly modify cellular behavior, resulting for example in uncontrolled growth and cancer; or -- in the most likely outcome -- it can produce a minute change in the genetic information, thus changing any of the thousands of gene products used by the cell. These effects tend to be proportional to dose, but they are extremely difficult to detect at the low dose levels occurring in the environment. Genetic research puts great emphasis on the development of sensitive detection methods. Such research is also, for the most part, multitechnological in its implications; although mechanisms of producing damage differ, the end results of mutagenesis are similar regardless of the specific inducing agent.

Our greatest capability for measuring genetic effects is in microorganisms. There is a pressing need to relate such effects to higher organisms and to develop methods of sufficient sensitivity and power to measure genetic damage directly in man.

Fig. 24. Relationship between cells, chromosomes, and nucleotides. If a pair of nucleotide bases weighs one unit, then a gene, which is about 60 turns of the DNA helix, weighs 600 units. By the same weight measure, the chromosome weighs 3×10^8 units and the cell 7×10^{12} units. The cell is microscopic; about 30 would stretch across the width of a pencil lead. The genome comprises the group of chromosomes within the cell that carry the complete set of genes for a particular organism.

injury occurs in other body cells, the defective information will be expressed in the injured individual only, not in his descendants. The most important manifestation of such somatic injury is cancer, but there may be other poorly understood consequences such as premature aging or organ failure.

During the past three decades – the heyday of molecular biology for simple organisms – molecular biologists have produced an exponential growth in our understanding of how the genetic system operates. However, they focused on viruses, bacteria, and fungi in which there are only one or a few chromosomes and in which nearly all the DNA consisted of genes coding for molecules of known function.

Molecular biology recently moved on to more complex organisms, beginning to lavish attention on the mammals and man himself. In looking at the genetic system of higher organisms, scientists immediately encountered the "DNA paradox." Although the visible increase in molecular complexity of these organisms was rather small and genetic data implied that the number of genes was not very much greater than in microorganisms, the size of the genome increased as much as a thousandfold! While some dismissed the excess DNA as nonfunctional – evolutionary garbage, perhaps – others maintained that it must have a purpose.

The answer is not yet in, but the balance tilts far more toward the latter view. First, on logical grounds the excess DNA should have some utility to the organism, however obscure. Energy is required to make DNA and chromosomes, and the evolutionary process of natural selection – an uncompromising form of

biological economics – should have eliminated any wasteful organisms. Second, as molecular study of the excess DNA was undertaken, it soon became clear that much of the DNA consisted of several specialized molecules. Although their functions still cannot be readily defined, their chemical properties are highly nonrandom and do not appear garbagelike at all.

The Kangaroo Rat

To learn the organization of DNA, we have intensively studied one of the specialized DNA types, known as satellite DNA, for the past several years.¹³ Through a combination of in-house biochemical research and a series of productive collaborations with investigators in Scotland, at UCLA, at the University of Colorado, and elsewhere, a broad picture of the molecular properties and chromosomal localization of several satellite DNAs has been gained. As has often occurred in biology, a particular species of animal with some unique features is providing information that illuminates a subject of general significance.

The kangaroo rat (Fig. 25) is the most successful and abundant mammal of the semi-arid and arid regions of North America. We had been studying specimens obtained on the Nevada Test Site in connection with the radioecology of tritium, when it was serendipitously discovered that their genome was the most extraordinary yet seen in the animal kingdom. In the principal species under study – *Dipodomys ordii* – more than half of the DNA in every cell nucleus consisted of satellite DNA. A model of this satellite DNA is shown in Fig. 25.

Fig. 25. Experimenters (left) examine a model section of satellite DNA for the kangaroo rat (right)

The most remarkable feature of satellite DNAs as a class of molecules is the arrangement of their nucleotide bases. They consist of subunits of three to perhaps 50 bases that are repeated in tandem, with minor variations, from 10^6 to over 10^8 times. This inordinate reiteration is not compatible with a large information content, so another type of function must be envisioned for satellite DNA. Indeed there is so far no evidence that this DNA interacts at all with the RNA interpreting subsystem.

We have studied 19 of the 22 or 23 species in the kangaroo rat genus; they contain the same satellites in amounts ranging from practically none to the 52% found in *Dipodomys ordii*.¹⁴ Clearly a kangaroo rat need not have satellite DNA to be a successful species. However, the quantitative variation in the amount of satellites among species should provide a means to vary the magnitude of certain other properties that might be functionally linked to satellite DNA. Correlations have, in fact, been found.

The karyotype of an animal is a specification of the number of chromosomes and the distribution of chromosome structures (basically the number of uniarmed and biarmed chromosomes). This can be abbreviated to the "fundamental number," which is the total number of chromosome arms and is characteristic of every cell in a given species of animal. Among kangaroo rat species there is a wide variation in karyotypes (Fig. 26) and fundamental numbers. These parameters are closely correlated with the amounts of certain satellite DNAs.

The most striking anatomical feature of kangaroo rats is their kangaroolike appearance (Fig. 25). Their large powerful hind legs are used for a jumping gait that can cover prodigious distances for the size of the animal; their rudimentary front legs are used for digging and transferring seeds to mouth or cheek pouches. One estimate of the extent of anatomical adaptation to this way of life is the relative lengths of their leg bones. Amazingly, such ratios as shown in Fig. 27 are correlated with the amount of satellite DNA. The correlation indicates that the low-satellite species are the more specialized for their environment, while the high-satellite species are more generalized.

One further correlation has been observed for each species of kangaroo rat. This is between the number of subspecies zoologists recognize on the basis of minor anatomical variations and the amount of satellite DNA. Similar to the previously cited anatomical correlation, the low-satellite species appear more specialized by exhibiting less variation in the form of subspecies,

Fig. 26. Two extreme examples of kangaroo rat karyotypes (a karyotype is a description of chromosomes for a given organism arranged in some meaningful order). (Top) Karyotype of a species with 70 uniarmed (acrocentric) chromosomes and two sex chromosomes. The fundamental number for this species is 70. (Bottom) Karyotype of another species with 70 biarmed (metacentric) chromosomes plus two sex chromosomes. The fundamental number for this species is 140. The karyotypes for other kangaroo rat species are intermediate, having a variety of uni- and biarmed chromosomal mixes.

while the high-satellite species appear more flexible through greater subspecies variation.

A limited survey of other rodent species outside the kangaroo rat genus appears to confirm the relationship between the number of chromosome arms and the amount of satellite DNA. More remarkably, at least one of the kangaroo rat satellites has been tentatively identified in several distantly related rodent species. This argues strongly for a significant biological function for satellite DNA, because the same simple sequence appears to have been conserved by natural selection ever since the several species diverged from a common ancestor over 50 million years ago. If the

Fig. 27. Shown here is a high correlation between amount of heavy satellite DNA and two indices considered by zoologists to be rough quantitative measures of adaptation for the kangaroo rat's jumping gait. (a) The Crural index gives the ratio of hind leg-bone length, tibia to femur. (b) The Intermetatarsal index gives the ratio of total length of main bones in the front leg to those in the hind leg. Dashed lines are fitted to data by least squares linear regression.

DNA had no function, it should have mutated into completely dissimilar sequences over that long a period.

A wide spectrum of information has been obtained about satellite DNA, particularly through the contributions of the kangaroo rat genus. This information points to a supragenetic role — a broad control function in the regulation of karyotype structure — which is linked to adaptations to the environment and the evolution of new species.

Applications to Man

Does this new knowledge of an exotic form of DNA gained from an exotic animal species have any relevance to man? Human DNA contains six to eight satellite fractions totaling about 5% of the genome. These satellites have chemical properties and specific chromosomal locations similar to those of rodent satellites, although base sequences are not yet known. There is very preliminary evidence that two kinds of chromosomal aberrations involving regions probably containing satellites are associated with reproductive failure and other human disorders. There is more abundant evidence in plants and animals that the distribution of injury to DNA by several chemical agents is nonrandom with respect to chromosome regions containing satellite and nonsatellite DNA. The overall importance of these observations is not clear, but there is adequate reason for further investigation.

Let us now place this form of highly specialized DNA in perspective with the other types of DNA found in the genome. An increasing proportion of the mysteriously abundant DNA of higher organisms is becoming easier to comprehend, in general terms at least. Five levels in the hierarchy of the genome can be defined:

- Unique sequences, including the genes coding for the structures of proteins and other specific sequences, are present in one or two iterations (copies) per cell. They represent about 50% of the genome.
- Repeated genes (less than 5% of the genome) code for types of RNA and chromosomal proteins, apparently because of transient requirements for rapid synthesis of the products.
- Moderately repetitive sequences (10 to 50% of the genome) are interspersed in several patterns with the coding DNA of the first two levels and may be involved in regulation of the activities of the RNA and protein-synthesis subsystems.
- Highly repetitive DNA (10 to 15% of the genome) other than that found in satellites has received little study.

• Satellite DNAs (5 to 50% of the genome) are the most repetitive sequences, highly variable in quantity, and located in massive tandem arrays occupying sizeable regions of chromosomes. The evidence concerning their function has been elaborated above.

Experiments involving injury to the genome by radiation and chemical mutagens or carcinogens give us some insight into the functions of the various levels. A mutation — as traditionally defined — involves injury to one structural gene, altering its information

content and resulting in synthesis of a protein product that is more or less defective. With cells in culture or small experimental plants and animals, we can determine the frequency with which particular genes are damaged by radiation or chemical agents. Using this mutation frequency and dose of agent, we can then calculate the size of the piece of DNA (referred to as the "target" volume) within which the damage occurred. In simple organisms this calculation shows that the place where the mutation occurs is, as expected, the same size as the known size of a gene.

However, much to our surprise, in organisms with genomes as much as a thousandfold larger than simple organisms, the target volume within which damage produces a specific mutation did not remain the size of a gene, as expected, but increased proportionately with genome size. For example, in radiation experiments a mouse, which has 200 times more total DNA than the bacterium *E. coli*, shows a mutation rate per gene 200 times greater than that of the bacterium. Figure 28 shows this relationship and indicates increased effect for a given amount of radiation with increased genome size for other selected organisms. Apparently, the sensitivity of the organism to genetic injury increases with the amount of DNA.

Because of experimental error, there is uncertainty about the absolute linearity of this relationship. But the major implication is that the effective size of the genetic unit for which a specific injury can be estimated in higher organisms not only includes the coding DNA (conventional concept of the gene) but also the last three levels of repetitive DNA described above. We hypothesize that genes and the other types of DNA, whose function was previously unaccounted for, must be functionally linked and together are subject to significant injury.

One must stand in awe of a genetic system that contains all the information needed to create and operate a human being within 6×10^{-12} g of DNA. But one must also be perplexed by the thousandfold "extra" parts. Is this apparent redundancy built in for flex-

Fig. 28. Relationship between genome size and amount of genetic injury produced by a unit of radiation (haploid genome refers to the amount of DNA in a simple organism or the amount in a sperm or egg cell of higher organisms). The data point for man is estimated. CHO means Chinese hamster ovary cell in culture. This relationship was discovered by four prominent American geneticists: S. Abrahamson, M. A. Bender, A. D. Conger, and S. Wolff [*Nature* 245, 460 (1973)].

ibility of coarse and fine tuning, or for superlative reliability and response to stress? Or is it an incredible "bureaucracy" that has figured out a way to escape its accountability to natural selection? Searching for the answers is a continuing challenge.

Key Words: biomedical research; DNA; RNA; DNA - organization; genome; kangaroo rat; radiation - biological effects.

FLOW KARYOTYPING: A PROMISING NEW CYTOGENETIC TOOL

We have developed a new and exciting method of chromosome analysis - flow karyotyping - that uses flow systems and is much faster than conventional

techniques. A companion method, flow sorting, provides chromosome populations purified to a degree not possible before. These two methods are providing new information about chromosomes and are potentially useful for detecting human chromosomal abnormalities, including those resulting from energy-related pollutants.

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Chromosomes are fundamental particles of biology in that they are the packaging units for the hereditary material of life, the genes. Genes consist of DNA and are the instruction book by which cells function and live. Precise measurement of the DNA in chromosomes has important applications in basic biology, in clinical research, in population genetics, and in monitoring human exposure to environmental contaminants.

Traditionally, cytogenetics – the study of chromosomes – has proceeded by visual inspection of stained cells on microscope slides, a useful but slow method. In May 1974, LLL used flow systems for the first time to measure and sort isolated chromosomes. This is an important extension of two ongoing aspects of flow systems development: flow cytometry, a key tool in biomedical research, which allows cell analysis at extremely high rates with statistical precision and sensitivity, and flow sorting, a technique that allows cells to be separated on the basis of their flow measurements.

Flow karyotyping is a new and exciting application of these flow systems. A karyotype is a description of the set of chromosomes in the cells of a given species. In flow studies, chromosomes are stained fluorescently for DNA and analyzed by flow cytometry to produce a DNA distribution – flow karyotype – unique to each species. Flow sorting provides purified populations of chromosomes for biochemical and biological study. We have detected abnormal chromosomes by flow-karyotype changes and are pursuing the technique as a potential means of monitoring human populations for cytogenetic alterations. Flow systems are a developing technology; much of our work centers on assessing their limitations and potential.

LLL Flow Cytometer

The LLL flow cytometer has been described in detail elsewhere.^{4,15} Briefly, a suspension of chromosomes released from about 5×10^6 cells is stained with ethidium bromide, a fluorescent dye specific to DNA. This suspension is made to flow in a very narrow stream so that the chromosomes travel single-file across a beam of intense blue laser light, which excites the dye to fluoresce. The fluorescent flash from each chromosome is picked up by an optical system and converted to an electrical pulse, which is amplified, digitized, and stored in the memory of a multichannel pulse-height analyzer. One thousand chromosomes are measured per second.

In the flow sorter the stream carrying the chromosomes breaks up into droplets shortly after the

fluorescent measurement. The droplets can be charged positively or negatively or left uncharged depending on whether or not the measurement of fluorescence falls within a preset range. As the droplets fall through a 1000-V field, they are deflected according to their charge and thus enter separate containers.

The result of analyzing a large number of chromosomes is a DNA distribution like that shown in Fig. 29 for the M3-1 line of Chinese hamster cells. The x axis represents chromosomal fluorescence, which is proportional to DNA content; the y axis is the number of chromosomes measured. For a typical flow karyotype we measure about 10^6 chromosomes, so the statistical precision is high.

M3-1 Cell Line Karyotype

Most of our work to date has been with chromosomes from the Chinese hamster M3-1 cell line. There are several reasons for this choice. To begin with, the M3-1 karyotype is relatively simple, containing only 23 chromosomes as compared with 46 chromosomes in a human cell. The M3-1 chromosomes are all well characterized, making the cell line a good model system with which to demonstrate the feasibility of flow cytometry. Finally, an isolated chromosome suspension was kindly supplied to us by Drs. Burki and Benz at the University of California, Berkeley, to help us get started.

Figure 30 shows an M3-1 cell line karyotype derived from photography and visual inspection of the chromosomes after they have been stained to reveal their banded structure. There are nine paired chromosomes (the members of each pair being essentially identical) and five unpaired chromosomes, for a total of 14 unique chromosome types. If the DNA content of each chromosome type differed from that of the others and if our analysis were perfect, then one would expect a DNA distribution with 14 peaks. The experimentally obtained distribution, however, has only nine peaks (Fig. 31). Thus some of the chromosomes have DNA contents too similar to be presently resolved with our flow cytometer.

The mean value of a peak reflects stain content and hence DNA content for chromosomes of that type. The area under a peak reflects the number of chromosomes of that type. For example, the two peaks (A and B) at high DNA values are due to the two pairs of large chromosomes (1 and 2) and have about equal areas. The smaller and unpaired number 5 chromosome produces peak D, which has only half the area of peaks A and B. Careful fitting and evaluation

Fig. 29. LLL flow cytometer: (bottom) laboratory setup; (top) operating principle and resulting DNA distribution, here for chromosomes of the M3-1 line of Chinese hamster cells. The chromosomes, stained with a DNA-specific fluorescent dye, flow single-file across the laser beam, which excites the dye to fluoresce. The result of analyzing some 10^6 chromosomes is a DNA distribution yielding the chromosomal fluorescence and thus DNA content (x axis) and the number of chromosomes measured (y axis). The silhouettes shown in the figure give the approximate size and shape of the chromosomes associated with each peak.

Fig. 30. Karyotype of the M3-1 Chinese hamster cell line (male) derived from conventional banding. Over 40 banded cells were examined to identify the rearrangements. Although not evident in these banding patterns, CYDAC analysis indicates that the pair in chromosome 1 differ in DNA content by about 6%.

Fig. 31. Flow cytometer data (top) and CYDAC data (bottom) on chromosomes from Chinese hamster M3-1 line cells. The points in the flow histogram are experimental data; the solid line represents a computer fit to these data. The length of the bars in the CYDAC plot indicates the number of chromosomes per cell and the location of the bars indicates their mean DNA content. The CYDAC data show a difference of 6% in DNA content between the two homologs of the number 1 chromosome pair. Normalization of the flow system and CYDAC results is based on the total DNA content of the karyotype; the differences of a few percent between flow-cytometer peak means and CYDAC means are probably due to staining and instrumental effects.

of the distributions indicate that they actually consist of two components: the peaks already described and a low continuum on which the peaks lie. This continuum is due to unstructured events such as chromosome clumps, debris, and broken or aberrant chromosomes.

We can verify this flow karyotype for the M3-1 cells with a second LLL analyzer, CYDAC, which measures the DNA content of chromosomes in an entirely different way. CYDAC (for cytophotometric data converter) is a microscope-computer hybrid that combines a visible-light microscope, special scanning electronics, and automated image processing. It operates by scanning the microscope field with a flying spot of light, defining (by automated image analysis) the exact boundary of each chromosome, and measuring the light absorbed by each chromosome's DNA. A special DNA-specific dye allows the process to be quantitative and because the measurements are made from cells on slides, each chromosome can also be identified morphologically.

The bottom part of Fig. 31 summarizes results of the CYDAC measurements for the M3-1 cell line. The x axis again indicates relative DNA content, and the y axis indicates the number of chromosomes per cell (either one or two). Table 2 compares these results quantitatively. The two methods agree well on both chromosomal DNA content and frequency. Hence we conclude that the DNA distribution produced by the flow system constitutes a legitimate flow karyotype.

To determine one measure of sensitivity of our flow system, we analyzed a subpopulation of cells grown

from an irradiated M3-1 culture. These cells exhibit a stable translocation involving the transfer of DNA between one of the number 1 and one of the number 4 chromosomes. Our analysis shows the expected loss of the two normal chromosomes and the gain of two abnormal chromosomes, one of which appears as a new peak in the valley between B and C. Furthermore, we can detect the abnormality when present in only 5% of the cells. If attainable with human cells, this 5% detection level would be a valuable tool to quantitate human mosaicism (an abnormal condition wherein a fraction of an individual's cells have a different chromosomal constitution).

Human Karyotype

These encouraging results led us to analyze human chromosomes from a cell strain derived from foreskin tissue. The human karyotype is far more complicated than the M3-1 chromosomes; there are 22 chromosome pairs plus the two sex chromosomes (X,X in the female, X,Y in the male). Our experimentally observed male DNA distribution from flow analysis is shown in Fig. 32a. Only seven peaks are resolved due to an overlap of chromosomes having very similar DNA content. Using the relative DNA content of human chromosomes as measured with CYDAC,¹⁶ Fig. 32b simulates a DNA distribution to be expected from flow analysis if we could measure the DNA content of each chromosome with a 4% coefficient of variation.

The simulated distribution is remarkably similar to our experimental data. Peak A is due to the number 1

Table 2. Quantitative karyotypes of Chinese hamster M3-1 cell line by flow cytometry (FCM) and scanning cytophotometry (CYDAC)

Peak designation	Chromosome designation	Relative DNA content		Number of chromosomes per cell	
		FCM ^a	CYDAC ^b	FCM ^c	Banding analysis
A	1	1.00	1.03	1.97	2
B	2	0.86	0.87	2.03	2
C	4, t(X:5)	0.55	0.54	3.26	3
D	5	0.47	0.45	1.03	1
E,F	6, 7, Y	0.36	0.36	4.88	5
G	8	0.30	0.29	1.99	2
H	9, M1	0.23	0.22	2.87	3
I	10, 11, M2	0.14	0.14	4.71	5

^aLargest relative DNA content = 1.00.

^bTotal relative DNA content of the karyotype via CYDAC equals the FCM total.

^cCombined areas of peaks A and B = 4.

Fig. 32. DNA distributions for human chromosomes: (a) determined experimentally with the LLI flow cytometer; (b) and (c) simulated from CYDAC data.

and 2 chromosomes, which are the largest and have very similar DNA contents. The remaining smaller chromosomes produce the peaks to the left. Peak C is due to chromosomes 9 through 12, which also have very similar DNA contents according to the CYDAC measurements. Peak G is due to the number 21, 22, and Y chromosomes, all of which have similar DNA contents.

We conclude, therefore, that our flow karyotype of human chromosomes is valid; however, for better resolution of the detailed structure of this complicated but important karyotype, we need to measure the chromosomal DNA content more accurately. As shown in Fig. 32c, with a 2% coefficient of variation the number of chromosome types resolved in our simulated distribution would increase from 7 to 18. In addition to better resolution, we may also need to measure more than the one parameter — DNA content — used thus far.

Improved Chromosome Recognition

There are several areas where flow-system technology can be improved to achieve better resolution. All of our measurements have been made on flow systems designed to analyze whole cells, not chromosomes. For high resolution, we need to be able to precisely illuminate the chromosomes; this requires that the chromosome be confined to within a few micrometres of the laser beam's center. At best, current systems confine the chromosomes to within about 10 μm . In addition, we need stable flow velocity, intense exciting light, increased efficiency in collecting the fluorescence, and laser lines that will excite the dyes at their absorptive maximum. Improved chromosome preparation and staining techniques are also necessary.

One concept we are investigating to improve our ability to recognize chromosome types is a flow system capable of scanning along the length of the chromosome. It seems feasible to produce a very narrow ribbonlike laser beam and to arrange the flow so that the chromosomes will travel lengthwise across the beam's narrow dimension. This dimension would be about 1.3 μm , with the chromosomes confined within 1.5 μm of the laser beam's center. Thus as each chromosome passes through the beam it will generate a time-dependent fluorescent signal representing the linear (axial) distribution of its DNA. Centromeres — the narrow joining regions — should produce dips in the DNA profile. Location of the centromere is a major distinguishing feature; hence this ability to recognize

centromeres would increase the number of resolvable chromosome groups and also aid in detecting aberrations.

In summary, our current flow cytometer can recognize seven groups of human chromosomes. Improving the resolution from 4% to 2% should reveal 18 groups and a centromere measurement would reveal 21 chromosome groups.

Applications

There are many areas of human cytogenetics to which flow systems can be applied. In aberration screening, for example, we need to detect and evaluate both homogeneous and heterogeneous chromosomal events. Homogeneous aberrations are present and identical in every cell; they represent the genetic characteristics of an individual and include changes in chromosome number (aneuploidy) and structure (heritable translocations). The need for their detection arises in prenatal diagnosis for birth defects, when evaluating spontaneous abortions, in neonatal screening, and when screening large populations for inherited anomalies.

Heterogeneous events are random from one cell to the next and are likely to have a low incidence per cell. These aberrations include dicentric (chromosomes with two centromeres), acentric fragments, and induced translocations. They are important when screening people who have been exposed to environmental contaminants or when testing physical or chemical agents for their ability to break chromosomes.

Homogeneous Events. The advantages of flow systems in analyzing chromosomal alterations can be seen by comparison with nonautomated conventional techniques. Any deviations from the normal number of chromosomes in a cell can pose serious problems. As an inherited alteration, chromosomal aneuploidy occurs with a frequency of a little over three per thousand live births.¹⁷ It is detected by counting the number of chromosomes, or the number of chromosomes of each type, per cell. Since the event is homogeneous, only a few (5 to 10) cells need to be analyzed.

If conventional chromosomal banding is used to identify the specific abnormal chromosome, a technician can analyze two to four cases per day (in a nonautomated mode). If we choose not to identify the particular chromosome by banding, this rate can be increased to 30 or 40 cases per day. Specific

banding information, however, considerably reduces the error rate.

High-resolution flow systems (i.e., a 2% coefficient of variation), coupled with centromere measurement, could substantially speed the analysis process. The focus would be on deviations from normal in the peak modes and areas of the flow karyotype. If 10^5 cells per person were analyzed, it should be possible to screen some 40 to 50 individuals per day. Although we might not be able to discriminate between some chromosome pairs, information that one of the two chromosomes is affected would demand a closer look by banding analysis. Thus, this sensitivity limitation is not serious.

Figure 33 compares simulated flow karyotypes for a normal human and for an individual with trisomy 21, an extra number 21 chromosome that causes Down's syndrome or mongolism. The simulations were made with CYDAC data and assume a 2% resolution. In the figure, chromosome 21 has the smallest amount of DNA and generates the leftmost peak; chromosome 22 generates the peak just to its right. The relative areas of the two chromosome 21 peaks differ as expected (2 vs 3). The relative heights of both sets of 21 and 22 peaks also differ and serve as a qualitative indication of abnormality. As a screening technique, flow cytometry could thus provide a quick and sensitive means of detecting homogeneous events.

Heterogeneous Events. Detection of unstable aberrations such as dicentric and deletions can also be improved with flow systems. The frequency of dicentric normally found in humans is 1 per 4000 cells; for isochromatid deletions (part of a chromosome missing), it is 13 per 4000 cells.¹⁸ By conventional analysis, a cytogeneticist would normally score from 100 to 1500 cells per sample, depending on the aberration frequency. On the average, one can score 200 to 300 cells per day, translating to an approximate rate of one case every five to seven days per technician.

Flow cytometry should considerably increase this rate. The procedure would be to look for an elevation in the flow karyotype's continuum or to count the number of centromeres in each chromosome. The requirements here are twofold: 1) highly reproducible chromosomal DNA distributions (i.e., both preparative and instrumental techniques must be stabilized), and 2) the ability to count the centromeres of chromosomes X and 1 through 12.

Preliminary results from our Chinese hamster work are promising. We have found that the coefficient of

Fig. 33. Flow karyotypes simulated for (top) a normal human and (bottom) an individual with trisomy 21 (i.e., an extra number 21 chromosome, resulting in mongolism). Both simulations assume a 2% coefficient of variation and no continuum in the distribution.

variation of measurement increases as does the area under the continuum when M3-1 cells are irradiated with x rays. The continuum's increase, as previously noted, reflects aberrant chromosomes in the population. Visual examination of sorted chromosomes shows that isochromatid deletions predominate at

small amounts of DNA and that dicentrics predominate at the large DNA amounts. Consequently, the continuum's area is proportional to the frequency of abnormal chromosomes. The lower limit detectable in these experiments has been about 0.15 aberrations per cell, or about 7 aberrations per 1000 chromosomes. Greater sensitivity is needed for practical use, but we feel we have made a good start in this direction and that the sensitivity can be increased.

Purified Chromosome Studies

Another important aspect of flow cytogenetics is its application to the study of chromosome structure. Each chromosome of the muntjac (an Indian deer whose cells have a very simple karyotype) has been purified to a high degree by flow sorting.¹⁹ For Chinese hamster chromosomes, four types are sufficiently unique in DNA content to be sorted separately.²⁰ With these pure chromosome fractions we can now, for the first time, probe the biochemical properties of DNA and protein at the level of individual chromosomes. We are looking to answer a number of questions. For example, are the satellite DNA sequences chromosome-specific in terms of quantity and type? Do proteins vary in quantity or type within chromosomes of a species or among similar chromosomes of several species? Can isolated chromosomes be useful for gene mapping? Answers to these and similar questions will provide basic biological information about the genetic system, will point the way to clinical applications, and will provide mechanism and assay possibilities for the evaluation of energy-related environmental mutagens.

Summary

The application of flow cytometry to chromosome analysis is an expanding field with much room for innovation and imagination. We are working to improve the sensitivity and speed of our flow systems. We are also exploring the feasibility of using flow karyotyping to screen chromosomal abnormalities and, ultimately, to monitor the effects on man of physical and chemical agents in the environment. New information on chromosomal structure and function should emerge.

Key Words: biomedical research; chromosomes; chromosomes - analysis; chromosomes - measurements; CYDAC; cytophotometry; DNA; DNA - measurements; flow cytometer; flow systems.

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