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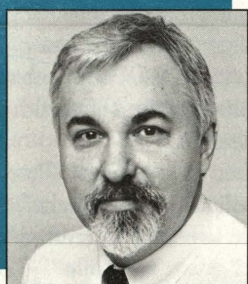
*Researchers develop new drugs
more suitable for liposome carriers.*

Clinician and chemist forge new approach to liposome research

Lab to Clinic



Roman Perez-Soler is an associate professor of medicine in the Department of Medical Oncology



Waldemar Priebe is an assistant professor of medicinal chemistry in the Department of Medical Oncology

Clinician Roman Perez-Soler, M.D., and chemist Waldemar Priebe, Ph.D., seldom meet formally. In their six years of researching liposomes and lipophilic anthracyclines, their best ideas most often arise in the hallway or over lunch in the cafeteria, a tendency that has held true since their first meeting in 1986. Priebe came to The University of Texas M. D. Anderson Cancer Center hoping to apply his knowledge of anthracycline synthesis to cancer treatment. He met Perez-Soler as a result of their mutual interest in new approaches to cancer therapy. Within an hour of their first meeting, Priebe had found his niche. "When Roman first described his work to me, I knew I could contribute," said Priebe, an assistant professor of medicinal chemistry in the Department Medical Oncology. "Combining the talents of a liposome expert and a medicinal chemist was a unique but natural project. We started to work immediately." Using their novel analogue-based approach, they have developed better liposome formulations and have created new doxorubicin analogues that, in preclinical studies, are more effective and less cross-resistant.

Doxorubicin difficult to encapsulate

Liposomes are fatty vesicles that form spontaneously when phospholipids are exposed to an aqueous solution. They have been extensively explored as carriers of different types of drugs to macrophage-rich organs, such as the liver and spleen. At M. D. Anderson Cancer Center, liposomes containing macrophage activators, antifungal agents, and cisplatin derivatives have been developed and tested in clinical trials during the last few years. However, for doxorubicin, one of the most effective anticancer agents, liposomal incorporation has proved a challenge. For over a decade, investigators have been examining various liposomal formulations for doxorubicin, but because it has a low affinity for lipids, getting doxorubicin into liposomes has been difficult, and even when the drug has been efficiently

encapsulated, the resultant liposomes have often been unstable. Typically, once injected, the liposomal doxorubicin degrades before reaching the tumor.

"Many people thought that it would be impractical to play with the drug to improve encapsulation, so they chose to focus on a wide variety of liposomal formulations," said Perez-Soler, associate professor of medicine in the Department of Medical Oncology. "At the time (1986), I wasn't particularly interested in liposomal doxorubicin, but I was interested in modifying drug pharmacology as a way of improving current anticancer therapy. When I met Waldemar, I said to myself, 'Here is an anthracycline chemist interested in application . . .,' and then things started to click."

Their initial strategy was to solve the encapsulation problem by focusing on better drugs rather than better liposomes. They began by studying how chemical modifications of anthracycline antibiotics would influence the drug's affinity for lipids, the fatty substance that composes the liposome carrier. Using his knowledge of anthracycline chemistry, Priebe would identify key areas in doxorubicin's chemical structure and synthesize new analogues accordingly; Perez-Soler would then formulate liposomes and test the formulation in animals.

When they began their research together, they had no specific idea of which modifications would work best. They have since studied about 40 such compounds. Surprisingly, they have found that increasing lipophilicity does not always increase encapsulation efficiency, as many in the field had thought. They have, however, identified three stable preparations whose encapsulation efficiency is over 98%. More important, one of these drugs, liposomal annamycin, was more effective than doxorubicin when tested in mouse tumor models.

Annamycin shows promise in preclinical trials

The drug's effect was initially studied in two cancers in mice: L-1210 leukemia and liver metastases of M-5076 reticulosarcoma. In L-1210 experiments, the cure rate

using doxorubicin was 0% with two different dose schedules, whereas with annamycin the cure rate was 50–80% (depending on the dose schedule used). In the M-5076 experiments, annamycin showed significant antitumor activity, whereas doxorubicin showed none.

Priebe and Perez-Soler's results, reported in 1990 in *Cancer Research*, were only the first step. They knew that developing effective clinical therapy would have to address the question of drug cross-resistance. Many tumors, though initially susceptible to a drug, eventually become resistant not only to the drug used but also to other drugs. This phenomenon, termed multidrug resistance (MDR), is being recognized as a significant obstacle to effective chemotherapy.

Although MDR most likely is the result of a complex array of biochemical mechanisms, one mechanism has been strongly implicated: drug efflux by the P-glycopro-

tein focus on the amino group. They therefore synthesized a new analogue, hydroxyrubicin, which was identical to doxorubicin except that the amino group is replaced with a hydroxyl group (Figure 1, p. 5).

Hydroxyrubicin experiments showed that the drug did not encapsulate in liposomes as well as annamycin did, so its utility in that area was limited. Nevertheless, it proved useful in counteracting MDR. In *in vitro* experiments comparing hydroxyrubicin with doxorubicin, hydroxyrubicin was more effective in inhibiting growth of resistant cell lines, had lower resistance indices in a battery of MDR cell lines, and showed higher levels of cellular uptake, indicating the drug was not being effectively bound and effluxed by P-glycoprotein. Moreover, in *in vivo* experiments in mice that had drug-resistant P388 leukemia, the analogue showed significant antitumor activity and was threefold less toxic than

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tein “pump.” Drug-resistant cells typically have shown high levels of P-glycoprotein. Presumably, P-glycoprotein binds to drugs and transports them out of the cell. How P-glycoprotein arises is not fully known, but it is believed that chemotherapy “selects” drug-resistant cells: The drug kills all cells sensitive to the drug, but those few that contain P-glycoprotein survive and proliferate. This would explain why a drug is effective against the primary tumor but ineffective against recurrences.

The next step: how to eliminate cross-resistance?

For Priebe and Perez-Soler, the logical next step was to develop a liposomal analogue that circumvented the P-glycoprotein pump. “The evidence implicating P-glycoprotein is highly suggestive, albeit circumstantial—a few studies have shown that MDR can be present in the absence of P-glycoprotein. Nevertheless, for our purposes, focusing on a way to counteract this mechanism was a reasonable objective,” Priebe said. They surmised that drug effectiveness and P-glycoprotein binding were controlled by different parts of the drug molecule. They were not sure which part of doxorubicin participated in binding to P-glycoprotein, but based on data in the literature and on their own studies, they decided to

the parent drug. Doxorubicin showed no antitumor activity in this test.

Removing amino groups may reduce cross-resistance

“We have some nice working hypotheses,” Priebe added. “Drugs that are typically thrown out of the cell by P-glycoprotein have an amino group. By removing it and preserving all other configurations, you can produce a non-cross-resistant compound. We’ve just received funding from the National Institutes of Health to do more studies along these lines.”

“So our research is not limited to liposomes,” Perez-Soler continued. “But because liposomes are a relatively easy drug carrier to use, we are very interested in pursuing all their possibilities.”

And it is such interest that is bringing the researchers, prompted by the intriguing results of the hydroxyrubicin experiments, back to the study of annamycin, which also has a hydroxyl group instead of an amino group.

“Using a study design similar to that of the hydroxyrubicin experiments, we are finding that

continued on page 5

Clinic will coordinate detection, screening, and study of this familial disease.

Neurofibromatosis: a new clinic brings new opportunities

Children with neurofibromatosis (NF), an often debilitating and sometimes fatal disease, face a difficult and uncertain future. Not only must they cope with a disease whose manifestations and clinical course cannot be predicted, they also must live with a higher than average risk of developing cancer. Moreover, because NF is a genetic disease, they carry a 50% risk of passing on the disease to *their* children, and they sometimes must watch a parent with a similar condition grow very ill. "Whenever you identify a child with NF," said Michael Needle, M.D., director of the Pediatric Neurofibromatosis Clinic at The University of Texas M. D. Anderson Cancer Center, "it is extremely important to look at the family as a whole."

Rare genetic diseases like NF require a systematic approach. Because the patient population is small, studies are relevant only if adequate numbers of patients are enrolled. Moreover, the patient's family members, who may also be at risk, must be identified, informed, and screened. Thus, a central location for studies and disease screening would make the provision of these functions easier. In the fall of 1991, just such an opportunity was created with the opening of the hospital's new Pediatric Neurofibromatosis Clinic. Needle, with help from Archie Bleyer, M.D., who heads the Division of Pediatrics, set up the clinic at M. D. Anderson Cancer Center, bringing together a multidisciplinary team of physicians and researchers who diagnose and treat many of the problems related to NF, including some cancers.

Disease characteristics are variable and progressive

NF, a disorder characterized by uncontrolled benign tumor growth, is caused by a gene mutation on chromosome 17. Only recently recognized as a number of different but related disorders, the most commonly identified form is NF-1, or von Recklinghausen disease. NF-1 affects as many as 68,000 Americans, striking equally both sexes and all races and ethnic groups. The disease, sometimes confused with the unrelated Proteus syndrome, or Elephant Man's disease, manifests itself in a variety of ways, including these three common ones: café au lait spots (similar in appearance to birth marks), neurofibromas (noncancerous tumors made up of nerve cells and the fibrous tissues surrounding these cells), and Lisch nodules (small, harmless lumps in the iris of the

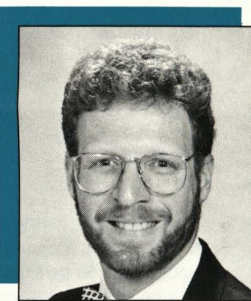
eye). In themselves, these three features cause few problems. In fact, as Needle explained, during their entire lifetime, some people will have only the café au lait spots and a few small, insignificant tumors. "Some people don't even know they have the disease," he said. "But when you diagnose NF in a child and start talking to the parents, sometimes the dad will just lift up his shirt and show you his spots. Until then, he had thought they were birthmarks." At the other extreme are those who have more significant problems from their first year of life, though this is uncommon. For most people who have NF, however, the problems start small and are generally progressive, worsening during puberty and early adulthood.

Problems are often age related

"When we screen children in the clinic," said Needle, "we are looking for a relatively well-defined, predictable set of problems." These include optic nerve gliomas, learning disabilities, scoliosis and other skeletal disorders, deafness, mental retardation, speech impediments, early or delayed puberty, high blood pressure, and itching. "You can almost take it by age group," he said of the signs and symptoms. In the youngest, below 3 years old, physicians often are simply confirming or denying a diagnosis of NF. They look at the family history and examine the patient for characteristic spots or tumors. In the 3- to 5-year-olds, the major concern is optic nerve glioma, a tumor that 10% to 15% of the children will develop. The tumor is low grade, slow growing, nonmetastatic, and rarely fatal, but it can take away vision. The glioma is occasionally treated with chemotherapy or radiotherapy. "We are pretty good at stopping the growth; maybe two-thirds of the time we can arrest the tumor's progression, but we can almost never reverse it."

The next biggest problem for this age group, according to Needle, is a 50% risk of having a learning disability. A large percentage of the children are hyperactive and have attention-deficit disorder; a smaller group has difficulty with visual-spatial relationships and visual-spatial abstract thinking. Some seem to have difficulty picturing things in their minds. "Another subset in the children," said Needle, "to me probably the most interesting of all, is a group that does very well *except* when it comes to expressing things on paper." When

Treatment Update



Michael Needle is director of the Pediatric Neurofibromatosis Clinic

“Our interventions are limited. Current treatment is only a small finger in the dike”

these children work with a computer or a video game, they do fine, but when asked to sign their names, they can't do it. “We have 14-year-olds who are illiterate,” he said. “Learning disabilities are overwhelmingly our biggest problem with young adolescents.”

Another very significant problem for children is the emotional load they carry. Children already coping with the trials of adolescence must learn to deal with lowered self-esteem because of the various cosmetic and health problems, anxieties over possible complications, and the daily frustration of dealing with the disease. Moreover, they often must watch in horror as the disease takes its toll on another, older family member. “One of our patients is a teenage girl whose mother died of NF, and she's very scared,” said Denise Long, RN, nurse coordinator of the clinic.

Adjustment problems are some of the most difficult for children

According to Needle, the clinic currently lacks the mechanism to look carefully at emotional problems such as these. And while it does provide many of the same types of services offered the hospital's cancer patients, one particular difference stands out. Whereas patients who have cancer come in every month or week and are sometimes admitted for several days, patients who have NF are seen much less frequently, sometimes only once for an initial evaluation. The ones with obvious problems, like the teenage girl previously mentioned, can be referred for counseling. “This child,” said Long, “is almost paralyzed by the thought that she's going to end up like her mother—dying. And though her tumor is benign, she does have a risk of developing cancer.” She and other children must learn to deal with that possibility.

Needle believes that once physicians and researchers have a better understanding of the fundamental molecular biology of NF, they can better address the psychosocial and physiological problems associated with the disease. “Right now,” he said, “we can recognize them and sometimes deal with them, but we can't reverse them, we can't cure them, and we can't prevent them. With some understanding of the molecular biology, we may be able at least to intervene in a much more directive fashion.”

Closing in on a gene

As a result of the new clinic, a ready source of tissue specimens is allowing researchers to establish a number of molecular studies related to NF. Louise Strong, M.D., chief of the Pediatrics Genetics section, is setting up a genetics laboratory dedicated to the study of NF. She said that genetics studies will help in identifying people at risk and in arranging genetic counseling for them. Eventually, the findings may lead to gene therapy. Meanwhile, researchers are hoping to learn more about the NF gene and its corresponding proteins.

The NF gene can be acquired in two ways: half the people inherit it from a parent and half obtain it as a result of a spontaneous mutation. Whether inherited or acquired, the gene confers the same risk of NF for children of these people: 50%. Vicki Huff, Ph.D., Department of Biochemistry and Molecular Biology, is examining patterns of spontaneous mutations by comparing tissue and tumor DNA samples of patients and relatives. She is also examining how the same gene mutation results in such diverse effects among family members, including malignant tumors in some but not others. It is possible that additional spontaneous (somatic) mutations may be the cause of these diverse effects.

Hideyuki Saya, M.D., Ph.D., and Toru Nishi, M.D., Ph.D., Department of Neuro-Oncology, have found two human NF-1 DNA transcripts. These transcripts, formed by alternative DNA splicing, produce different NF-1 proteins that may be related to the development of tumors in NF-1 patients. In addition, these transcripts may be important in the development of neuroectodermal brain tumors in non-NF-1 patients, said Victor A. Levin, M.D., chairman of the department. Two clinical trials using retinoic acid to alter the relative amounts of these NF-1 transcripts in brain tumors are currently under way, and a study of NF-1 patients is being contemplated.

Researchers see many long-term goals for the clinic

Though the clinic is still quite new, those associated with it are already enthusiastic about its future and express a number of long-term goals. The hope is to develop better ways of screening patients for major problems, to detect those problems earlier, and to

Liposome Research

continued from page 2

annamycin's uptake—and consequently its cytotoxicity and lack of cross-resistance—is much higher than that of hydroxyrubicin," said Priebe. Consequently, annamycin, because of its high incorporation, increased efficacy, and non-cross-resistance, may be a promising candidate for clinical trials.

The implications of Priebe and Perez-Soler's research are far reaching; creating stable, tumor-targeted liposomes that contain effective, non-cross-resistant compounds would be a major advance for chemotherapy. However, they caution that attaining such a goal is still in the future. They continue to work on novel liposome formulations and non-cross-resistant

analogues and to study mechanisms of MDR and drug interaction with biological membranes—a collaboration they obviously enjoy.

"We both learn a lot from each other," said Priebe, to which Perez-Soler readily nods. As they leave the interview and walk down the hallway, one notes that they immediately begin a rapid-fire exchange of words. One gets the impression that, before they reach the elevator, yet another idea will be born.

Physicians who desire additional information may write Roman Perez-Soler, M.D., Department of Medical Oncology, Box 80, or Waldemar Priebe, Ph.D., Department of Medical Oncology, Box 41, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, or call (713) 792-6363 (Perez-Soler) or 792-3777 (Priebe). ■

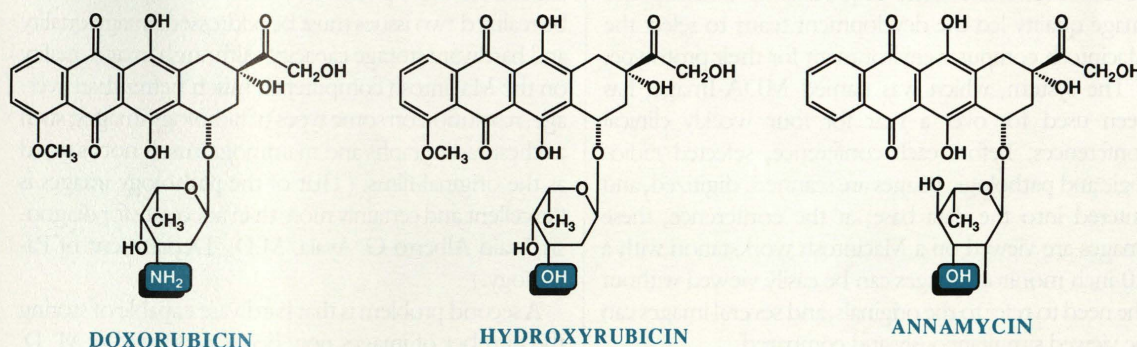


Figure 1. Chemical structure of doxorubicin and two of its analogues, hydroxyrubicin and annamycin. In the analogues, the amino group (NH₂) is replaced with a hydroxyl group (OH).

provide improved patient care, including coordinated, consolidated services. A common problem for NF patients is being treated for one thing by one doctor and for another by a different doctor. "Very often, no one puts the whole puzzle together," said Needle. "Tumors, bone problems, learning disabilities—they are diverse concerns, and the person treating one problem may not think about the other possibilities."

Another, related, goal is closer coordination between the pediatric clinic and the Adolescent and Adult Neurofibromatosis Clinic in the Department of Neuro-Oncology. Adult patients have a different set of problems from children, explained Lesley K. Newton, M.D., director of the clinic. The focus for adults is more on neurological impairment and malignancies, including neurofibrosarcomas and benign and malignant brain tumors. The two clinics, though closely associated, are administered independently. The hope is to eventually find space where they can operate side by side.

According to Needle, the major long-term goal is clearly prevention. "The truth is," he said, "our interventions are limited. Current treatment is only a small finger in the dike." Needle admitted that most research is related to diagnostic measures and has not yet translated into intervention. "The point of the clinic," he said, "is not to wait for kids with NF to present in disaster, but to catch up with them early enough while their problems are small, and ultimately, with the help of the molecular biologists, to see how many of the problems we can prevent altogether."

Physicians who desire additional information may write Michael Needle, M.D., Pediatric Neurofibromatosis Clinic, Department of Pediatrics, Box 87, or Lesley K. Newton, M.D., Adolescent and Adult Neurofibromatosis Clinic, Department of Neuro-Oncology, Box 100, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, or call (713) 794-5403. ■

Computerized Network

continued from page 8

divisions of Diagnostic Radiology and Pathology and the Section of Melanoma/Sarcoma of the Department of Medical Oncology. Its first goal was to create a prototype online "teleradiology/pathology" system to be used for multidisciplinary clinical conferences. The planners required that the system have the ability to capture and display radiographic and pathologic images from anywhere in the institution and be expandable to meet the needs of all clinical conferences held at M. D. Anderson. The system also had to be simple to operate by beginning computer users, require minimum software development, and be low cost; it also had to have color capability, as pathology images must be in color to be effective. These latter requirements and superior image quality led the development team to select the Macintosh computer environment for their prototype.

The system, which was named MDA-Image, has been used for over a year for four weekly clinical conferences. Before each conference, selected radiologic and pathologic images are scanned, digitized, and entered into the data base; at the conference, these images are viewed on a Macintosh workstation with a 20-inch monitor. Images can be easily viewed without the need to refer to the originals, and several images can be viewed simultaneously and compared.

These conferences have generated a high level of interest in the MDA-Image system among the participating medical staff. Said Mark E. Moffitt, now Director of Diagnostic Imaging Information Services, who has been involved in the project since its beginning, "the real goal of the initial project was simply to develop the capability to put these images on an electronic system.

Since then, the staff have proposed lots of different applications."

Technology still in developmental stage

The staff's enthusiastic proposals, however, raise the question of the technology's current capabilities. Several imaging equipment vendors have expressed interest in supporting further development; clearly, they see many potential commercial applications in this system, such as "turnkey" (i.e., ready-to-use) computer systems for hospitals and diagnostic centers, interactive systems for conferences among multiple institutions, and reference and teaching materials, such as atlases, clinical conferences, and proceedings. There are no standards now in place for these systems; the companies may find M. D. Anderson's system particularly attractive because of its unique pathology image capabilities.

Such interest by commercial vendors demonstrates the technology's potential, but before its potential can be realized two issues must be addressed: image quality and hardware storage capacity. Although image quality on the Macintosh computers is much better than average, resolution on some types of radiology images, such as chest radiographs and mammograms, is not as good as the original films. (That of the pathology images is "excellent and certainly more than adequate for diagnosis," said Alberto G. Ayala, M.D., Department of Pathology.)

A second problem is that hardware capable of storing the number of images now being generated at M. D. Anderson is too expensive; for now only selected images, not the entire patient file, can be stored. The availability of only selected images makes most radiologists nervous; they need to see all the images to make the best decision. Although the eventual goal is to store all images in the MDA-Image system, to do so now would require six to ten storage units each the size of a small

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“Within about 10 years, all of our images will be stored electronically, without need for slides or films”

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room, according to Moffitt. John E. Grossman, Project Director, Imaging and Software Systems, pointed out, however, that “data storage technology is improving quickly, and its cost is going down. It may be possible within 10 years for all of our images to be stored electronically, without need for slides or films. Every day there’s something new; it’s gotten to the point that a lot of the big players—IBM, Kodak, GE—are interested in this area.” With such commercial interest, larger capacity systems should not be far behind.

Radiologic-Pathologic Institute planned

Wallace acknowledges that MDA-Image is still in a developmental phase. He and other faculty involved in the project are already planning new applications and new methods of supporting project development. In addition to the clinical conferences, “another big motivator for the project,” according to Grossman, was an anatomical atlas developed by William R. Richli, M.D., which brings together CT and MRI images, high-quality photographs of gross specimens and thin-tissue slices (which can be viewed from many different perspectives), and explanatory text. Richli’s early version of the atlas is now being expanded; the eventual goal is to cover the entire human body. The faculty’s most ambitious suggestion is the creation of the Radiologic-Pathologic Institute, a diagnostic reference and teaching center within M. D. Anderson. The institute would both develop the image data base technologies needed to create a universal electronic imaging system and become a reference center for diagnostic images, offering various training materials for anatomy, radiology, pathology, and diagnostic oncology. They also envision that the institute would become a center for training in those fields, by developing new teaching methods that incorporate the electronic image system.

Now that the MDA-Image system is operational for the conferences and has at least two ongoing projects (that is, expansion of the image data base and the anatomical atlas), the first step toward achieving the goals of the Radiologic-Pathologic Institute, the creation of the Texas Outreach program, has already been taken. The Texas Outreach program, which was funded by a grant from the Physicians Referral Service of M. D. Anderson, will expand the MDA-Image system’s capabilities to allow sharing of electronic images with other institutions and physicians outside M. D. Anderson. A

group of physicians in the Rio Grande Valley of Texas (as yet undetermined, but perhaps connected with Pan American University) will probably be the first outside node in the MDA-Image network. From a workstation placed at their location, they will be able to both send images to and receive them from M. D. Anderson and confer with M. D. Anderson specialists on patient decision making. The system will also allow transmission of educational materials on diagnostic oncology. Eventually many Texas physicians, including those in private practices, hospitals, and industrial settings, may be able to use this network. Expansion of the network is dependent on the continuation of funding or development of other funding sources.

Educational applications of MDA-Image

Another proposed project is a series of specialized educational CD-ROM products to be derived from the image data base; examples are a “textbook” on gynecological oncology and the proceedings of various clinical conferences. CD-ROM not only offers illustrations far superior to those of printed works, but also has an interactive capability that allows the user to review the information in the sequence he or she prefers. Of course, the CD-ROM format also offers authors the opportunity to present their knowledge and skills in a new, dynamic way, and many are eager to respond to this challenge.

The final plan for the MDA-Image system is its incorporation into an expanded training program in diagnostic oncology offered by the Radiologic-Pathologic Institute. The institute’s planners envision a true academic institute that will host physicians and researchers from all over the world who want to take advantage of state-of-the-art training in the field. Here at M. D. Anderson they will learn to take advantage of computer technologies to enhance their skills in cancer diagnosis.

Physicians who desire additional information may write Sidney Wallace, M.D., Department of Diagnostic Radiology, Box 57, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030, or call (713) 792-2714. ■

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Inside

Novel approach to liposome research
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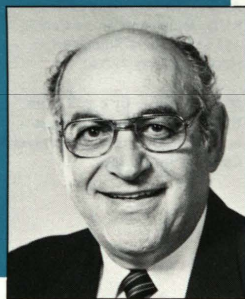
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*Radiology and pathology images
transmitted by computer.*

Computerized network enhances interdisciplinary patient management

Technology



Sidney Wallace is chairman
of the Department of
Diagnostic Radiology

A new computer application developed at The University of Texas M. D. Anderson Cancer Center could have far-reaching effects on the way radiology and pathology images are stored, retrieved, and used. Such images play a critical role in the diagnosis and staging of cancer and the management of cancer patients. Sidney Wallace, M.D., chairman, Department of Diagnostic Radiology, has estimated that the divisions of Diagnostic Imaging and Pathology at M. D. Anderson Cancer Center generate about 15,000 images each day. Several hundred images may be generated for a single patient. Simply keeping these images organized and accessible is a major endeavor.

Traditionally, radiologic images have been stored in large folders on shelves in cavernous storerooms located far from the clinics and research areas where they are used. Distributing, collecting, and filing these folders is a labor- and time-intensive process. Files may become lost, misplaced, or physically damaged. Large hospitals

and research centers that generate thousands of these images have long sought better systems for storing and retrieving them.

Likewise, storage and retrieval of pathology images can pose a problem, but for a different reason: each pathologist may be responsible for or may prefer developing his or her own cataloging and filing system. Some institutions have systems in place for shared filing systems, but retrieval may rely heavily on the memory and knowledge of the individual who worked with the slides. Many pathologists have recognized the need for a shared-access system for storage and retrieval of pathologic images.

Electronic system improves access to images

Development of an electronic system that would handle both types of images began at M. D. Anderson in 1989. The project was undertaken jointly by the

continued on page 6