Biochemical Markers: Early Warning Signs of Cancer

The importance of early detection of cancer is difficult to overemphasize. With current detection techniques, more than half of all cancers develop life-threatening metastases (that is, spread throughout the body) before they are first detected. If tumors could be detected while they are still small and localized, some scientists estimate that as many as 90 percent of all cancers could be cured, compared to the 35 percent that are now cured. It is not surprising, then, that large numbers of investigators have been seeking simple biochemical and immunological tests that can reveal the presence of cancer long before clinical signs of the disease become apparent.

The search for biochemical markers began with agonizing slowness. As recently as 3 years ago, there were only two important candidate biochemical markers, and neither of them seemed useful for screening (*Science*, 12 April 1974, p. 147). Since then, however, the pace of discovery has accelerated dramatically, in part because of the development of more sophisticated analytical techniques. One measure of the current interest is the fact that the National Cancer Institute (NCI) has sent out coded samples of blood or urine from cancer patients and healthy individuals to more than 150 investigators who thought they had found specific tests. Distressingly, the results obtained in the overwhelming majority of these tests were no better than could be expected from chance. Nonetheless, some markers are promising. One biochemical marker is now being tested as a screen for prostate tumors, several are being used more or less routinely at a few institutions to monitor the course of cancer therapy, others seem, in preliminary experiments, to be specific markers for individual tumors, and still others have been found to be indicators of the presence of cancer.

The markers that were prime candidates 3 years ago are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). CEA is now used routinely at some institutions for monitoring therapy of several different types of tumors (see box). AFP has been used in the People's Republic of China to screen more than 500,000 individuals who have a high risk of developing liver tumors. It has also been used in this country for monitoring therapy of tumors of the testicles and as one of several markers employed in prospective screens. CEA and AFP are prototypes of the so-called oncofetal antigens, glycoproteins and other substances that are present in tumors and in fetal tissues but are not present or are present only in very small quantities in healthy tissues. Other categories of materials that have been suggested as potential markers include abnormal enzymes, hormones, and nucleosides produced by tumor cells, antigens from their surface, and antibodies to them.

Some Markers Linked to Specific Tumors

The best example of the type of marker sought by investigators is an abnormal enzyme, acid phosphatase, found in the blood of patients with prostate tumors. T. Ming Chu and his associates at Roswell Park Memorial Institute developed a simple, inexpensive (about $3), and specific immunologic test that can detect the enzyme in less than a drop of blood. Chu's preliminary results indicated that the test can detect as many as half of prostate tumors while they are still localized and as many as 80 percent of the more advanced cases. This is a great improvement over the roughly 30 percent detection of advanced cases with existing techniques. Most important, preliminary results indicated that the test gives very few "false positives"—that is, it does not indicate the presence of a tumor when there is none. A very low incidence of false positives is a necessity in any test that might be used for screening.

Chu's test is now undergoing intensive study at more than 25 cancer centers around the country in a major study sponsored by NCI and the American Cancer Society. If this study is successful, the test will probably be used for routine screening of men who have a high risk of developing prostate cancer, particularly men over the age of 55. The 5-year survival rate for prostate cancer is more than 80 percent if the disease is detected while it is still localized; wide use of the test could thus make a sharp reduction in the number of men (20,000) who now die of the disease each year in this country.

A second test that may get wide use soon was developed by Phillip W. Banda and Marsden S. Blois of the University of California at San Francisco for the detection of melanoma, a highly malignant tumor of pigment cells in the skin. Their assay depends on the fact that the urine of melanoma patients contains higher than normal concentrations of many by-products of the pigment melanin. They have found that these by-products can be separated from the urine by high pressure liquid chromatography and detected with a specific colorimetric agent.

The technique may not be sensitive enough to detect small melanomas, but that is generally not necessary since melanomas are visually obvious and can easily be detected by a physician or by the patient himself. Its main value, Banda says, is for determining whether the primary tumor has metastasized, monitoring the course of therapy, and detecting hidden recurrence and spread of the disease. Blois and Banda's results indicate that the test misses fewer than 20 percent of metastases (false negatives) and has an even lower rate of false positives. The two investigators are providing a monitoring service, upon request, for melanoma-patient urines provided by physicians and hospitals in the San Francisco Bay area.

There are a small number of other candidate markers for which results suggest specificity for individual tumors. Thomas S. Edgington and his colleagues at Scripps Clinic and Research Foundation have, for example, isolated a glycoprotein that they have observed so far only in human breast tumors and in human breast cancer cells maintained in tissue culture. They are now attempting to develop a more sensitive assay to determine whether the glycoprotein is present in the blood of breast cancer patients.

In similar fashion, Chu and his colleagues have isolated a glycoprotein that they believe to be specifically associated with tumors of the pancreas. Robert L. Hunter and his associates at the University of Chicago have isolated a different glycoprotein from pancreas tumors and from fetal pancreases. And Malaya Bhattacharya of Roswell Park has isolated two glycoproteins that she believes are specifically associated with tumors of the ovaries. A great deal more work will be required with each of these tests, however, to determine their precise specificity and sensitivity.

Most other biochemical markers for cancer are, like CEA, found in association with more than one type of tumor and, often, in association with other, nonmalignant conditions. One of the best examples of such a marker is the so-called B-protein isolated by Edsel T. Bucovaz and his associates at the University of Tennessee. They have found

that a protein from yeast, known as coenzyme A synthesizing protein complex, binds specifically to a protein found in the blood of cancer patients. They quantify the amount of B-protein in a subject's blood by labeling the yeast protein with a radioactive isotope.

In tests performed on blood samples from more than 4600 patients, Bucovaz has obtained false negatives in fewer than 8 percent of the patients and false positives in less than 9 percent. Many of the false negatives were obtained in patients with breast or skin cancers; a much lower rate of false negatives was

CEA: Puzzling New Information About a Useful Marker

Carcinoembryonic antigen (CEA) was first isolated from colon tumors in 1965 by Phil Gold and Samuel O. Freedman of the McGill University School of Medicine. It was originally thought to be a specific marker for colon tumors, but subsequent work by a large number of investigators has shown that elevated concentrations of CEA also appear in the blood of patients with many other types of tumors and of healthy individuals who smoke heavily or who have certain other nonmalignant conditions.

CEA thus does not represent a practical screen for cancer. But preliminary results suggested that it has great value for monitoring the course of cancer therapy, and 3 years ago the Food and Drug Administration licensed Hoffmann-La Roche Inc. to manufacture and sell a radioimmunoassay kit for that purpose. CEA is the only biochemical marker for cancer to receive this approval and thus has been studied much more extensively than any other such marker. A recent conference at the University of Kentucky at Lexington provided much evidence justifying the initial claims for the usefulness of CEA. Some new evidence also suggests that CEA may somehow be a marker for an unidentified virus.

Several investigators have shown that CEA concentrations in the blood decline if the cancer therapy produces a regression and rise again later if there is a recurrence of the disease. A. Munro Neville and his colleagues at the Royal Marsden Hospital in Sutton, Surrey, England, for example, found that rising levels of CEA predicted a recurrence of tumors of the breast, colon, and rectum more than 70 percent of the time. The CEA levels generally rose 9 to 12 months before other clinical signs of the disease became apparent.

Putting these and other similar findings into practice, John P. Minton and his colleagues at the Ohio State University College of Medicine have performed second-look surgery on patients who had had successful surgery for colon cancer and later developed a rising concentration of CEA. They found a recurrence of the disease in nearly all the patients they examined. They also found that, if they relied on rising CEA levels even in the absence of other indicators, they could locate tumors while they were still localized and removable in 75 percent of the cases; if they waited until other evidence was available, a much lower percentage of tumors were removable. Minton seems to be the most optimistic investigator regarding this use of CEA, but others have obtained similar results, and the Society of Surgical Oncology is now planning a nationwide trial of second-look surgery in colon cancer patients.

Even more interesting are some of the findings about CEA itself. CEA was once thought to be a single substance that just happened to be found in a variety of conditions. Now, work by Gold and other investigators, such as Elliott Alpert of the Massachusetts General Hospital, indicates that CEA is actually a family of glycoproteins that appear to share one or more antigenic sites that are recognized in the CEA assay. In essence, this suggests that there may be CEA-like antigens that are specific for individual tumors, but that current tests are not specific enough to distinguish between them.

Support for this possibility was provided by Thomas S. Edgington of the Scripps Clinic and Research Foundation. He used a highly purified species of CEA from gastrointestinal tumors to develop a more specific, second-generation test that he calls CEA-S. This test detects about the same percentage of gastrointestinal tumors as does CEA, but it produces far fewer false positives. As just one example, elevated concentrations of CEA-S were found in 0.31 percent of patients with nonmalignant diseases, compared to the 9.4 percent who had elevated concentrations of CEA. It thus seems possible that CEA tests can be developed that might show much more utility for screening.

Other aspects of CEA are much more puzzling. Hoda A. Guirgis and her associates at the Creighton University School of Medicine have studied CEA levels in families that have a high incidence of tumors of the colon, lung, or breast. They found higher than normal concentrations of CEA in the immediate relatives of cancer patients. The children and the siblings of these patients demonstrated high levels of CEA, but the highest levels, suprisingly, were found in the spouses of the patients. This result indicates that the epidemiology of cancer in these patients is exceptionally complex. It also suggests—but certainly does not prove—that there may be some form of transmission of an infectious agent.

Support for this tenuous link between CEA and an infectious agent comes from Gary L. Gitnick and his associates at the University of California at Los Angeles. Gitnick examined 486 samples of blood in a blood bank and found that 50 of them contained higher than normal concentrations of CEA. Among the 50 recipients of this CEA-positive blood, 38 who had previously exhibited normal CEA concentrations developed persistently high concentrations of CEA. Seven of these returned to normal in 2 to 3 months, but eight became chronic carriers of CEA and 23 developed hepatitis. In contrast, all of 50 carefully matched controls who received CEA-negative blood continued to show normal concentrations of CEA and only six developed hepatitis.

In a separate study of patients whose hepatitis was not caused by either the hepatitis A or B viruses (non-A, non-B hepatitis), Gitnick found that 62 percent had high concentrations of CEA. This is by far the greatest incidence of high CEA concentrations that has been observed in a nonmalignant disease. Both results again suggest that CEA is somehow indicative of a virus. Gitnick's results are very recent, however, and no one has yet had a chance to confirm them. But the high incidence of hepatitis among patients who receive CEA-positive blood provides good argument for a much more intensive study of this phenomenon.—T.H.M.

obtained in patients with tumors of internal organs, which are generally harder to detect by other means. The largest number of false positives occurred with blood from burn patients and women in the third trimester of pregnancy. Bucovaz has filed a patent application for the assay and is now trying to arrange a much larger test of its efficacy.

Another general blood marker has been discovered by Robert G. Parsons and his associates at Scripps. They find that a protein that binds to DNA is present in much higher concentrations in the blood of patients with leukemias, lymphomas, and melanomas than in healthy individuals. The protein is apparently a degradation product of a component of the immune system known as complement 3 and is thus named C3DP. They have shown so far that the concentration of C3DP correlates with the severity of the malignancy in the majority of these cancer patients and that it is a useful marker for monitoring therapy.

Carbohydrates bound to proteins in the blood may also serve as markers for cancer, according to a group headed by Joseph F. Weiss of the Armed Force Radiobiology Research Institute and Paul B. Chretien of NCI. They have found that the concentrations of sialic acid, hexoses, hexosamine, and fucose are all higher in cancer patients than in healthy individuals. The increased concentrations correlate with the severity of the disease. These compounds appear to be bound to glycoproteins found in the α-globulin fraction of blood serum. The group is now studying use of these glycoproteins to monitor therapy of a variety of malignancies. They have also found an inverse correlation between serum levels of certain glycoproteins and immune status. These results agree with previous findings by other investigators that high concentrations of some glycoproteins may suppress immune activity.

Hormones in the blood may also be nonspecific markers. Many investigators have, for example, found the pregnancy hormone human chorionic gonadotropin (HCG) in association with various malignancies and it is frequently one of the markers used in multiple assays. Studies by Griff Ross of NCI and by others have shown that HCG is particularly useful for monitoring therapy of choriocarcinoma. K. Robert McIntire of NCI has also shown that it is useful for monitoring testicular tumors. Armand Tashjian of Harvard Medical School and others have shown that high concentrations of calcitonin are associated with several types of tumors and can be used to monitor therapy of medullary carcinomas of the thyroid. And Rosalyn S. Yalow of Mt. Sinai Hospital in New York City has found high concentrations of a precursor of adrenocorticotropic hormone (ACTH) in many patients with lung cancer.

Compared to the search for markers in the blood, the search for markers in urine has been much more limited. This seems rather surprising because, according to Daniel Rudman of the Emory University School of Medicine, most patients with disseminated cancer excrete as much as a gram of protein in the urine each day. The healthy individual excretes little or no protein, but some patients with kidney diseases excrete normal blood proteins with masses higher than 70,000 daltons. Cancer patients, Rudman says, excrete unusual proteins that have masses ranging from 10,000 to 60,000 daltons.

Rudman and his colleagues have so far isolated and purified five of these proteins and developed assays for them. They have found that one or more of the proteins appear in the urines of as many as 70 percent of patients with disseminated cancers and in a smaller percentage of patients with localized disease. They have also found that the amount of protein in the urine is a good indicator of the efficacy of chemotherapy for tumors of the head, neck, and lung.

Another class of materials found in urine is polyamines, such as spermidine, putrescine, and spermine, which are thought to be by-products of the increased concentrations of RNA that accompany cellular growth and proliferation. Diane H. Russell and her associates at the University of Arizona first reported high concentrations of polyamines in the urines of as many as 70 percent of cancer patients in 1971, and their work has been confirmed and extended by many investigators. It now appears that changes in the concentrations of polyamines in urine and other biological fluids are useful for monitoring chemotherapy in more than half of patients with Burkitt's lymphoma, melanomas, cancers of the blood, and tumors of the colon, rectum, and brain.

Most of the discoveries of the biological markers now under consideration have been serendipitous; the specificity of the markers is still undetermined because no one knows for sure what substances should be in the blood. This situation may change soon. Norman G. Anderson and his associates at Argonne National Laboratory have developed an automated, high-resolution electrophoresis system for separating the large number of proteins and glycoproteins in biological fluids. By looking at samples from large numbers of healthy individuals, Anderson plans to produce a catalog of all the proteins that are normally present. He has already cataloged most of the proteins in blood serum and is now studying erythrocyte proteins.

When Anderson is finished with the cataloging, he will make a survey of fetal tissues and tumors to determine what proteins are present. He thinks that it should be possible to find a whole spectrum of specific biochemical markers.

Use of Multiple Markers

Until such highly specific markers are developed, however, many investigators think the best results in screening can be obtained with a battery of assays. Paul Franchimont and his colleagues at the University of Liège in Belgium, for instance, use a battery that tests for five substances in the blood—CEA, AFP, HCG, a subunit of HCG, and kappa-casein, a phosphoprotein of human milk. In tests on more than 1450 individuals, they found that the battery detected 72 percent of the cancers and had a false positive rate of about 15 percent. Most of the false positives occurred in patients with hepatitis or cirrhosis of the liver, conditions in which tissue regeneration produces some of the fetal proteins.

Franchimont argues that the sensitivity of the test can be increased to 85 to 95 percent by inclusion of two other assays—one for another oncofetal protein and one for fragments from a virus. He plans to test the efficacy of the enlarged screen by monitoring some 500,000 workers in the Liège area. Many of these are workers from metallurgic and radioactive isotope industries who might be expected to have a higher than normal incidence of tumors.

A few other groups of investigators are also using batteries of assays, some analyzing for as many as 18 different markers, but few are as optimistic as Franchimont about the prospects for screening. Most would probably agree with McIntire, however, that use of multiple markers when a tumor is first suspected provides the best opportunity to find one or more markers for monitoring the course of therapy and detecting recurrences of the disease. Most would also agree that the use of multiple assays will continue to increase in the future and that the incorporation of newer assays will increase their efficacy. It may not yet be possible to recognize the presence of a tumor and identify its site by means of a simple blood test, but it now seems much more likely that such a day will eventually arrive.—THOMAS H. MAUGH II

Erratum: The cost of semiconductor grade silicon was incorrectly stated as $65 per ton (29 July, p. 446). The correct figure is $65 per kilogram.