Biological Markers for Chemical Exposure

Effects on reproduction and chromosomes may be the best indicators of exposure to chemicals in dumps, but the health effects are uncertain

When the cap on the chemical wastes storage site at Love Canal was disrupted by construction activities, toxic chemicals leaked to the surface of the soil and into the basements of nearby homes. It was a relatively easy matter to identify the principal chemicals present and to measure their concentrations. It was far more difficult to determine which residents of the area, if any, had been exposed to the chemicals and, if so, to which ones and for how long. Even today, that determination has still not been made to anyone's satisfaction.

The problem is not restricted to Love Canal; it is common to all hazardous waste storage and dump sites. Even when it is known that toxic chemicals are present in air or water, it is still difficult to obtain an objective measure of the extent of exposure. Serum and urine analysis are frequently of limited value because most chemicals are cleared from these fluids rather quickly. Analysis of adipose tissue, human milk, and hair can be of use in documenting exposure to chemicals that are fat-soluble. What is needed is a more general indicator of exposure, ideally one that does not require analysis of specific chemicals.

Three recent meetings were convened* to consider the problems associated with hazard waste storage and dump sites and to evaluate ways to monitor exposure to toxic substances (*Science*, 29 January, p. 490). Among the chief subjects of concern were chromosomal damage, reproductive alterations, and neurotoxic effects of exposure. The consensus seems to be that these deviations can provide ways to monitor chemical exposure, but that it is extremely difficult to relate that exposure to long-term health effects.

One possible way to assess exposure to toxic chemicals is to study chromosome aberrations and sister chromatid exchanges. Chromosome aberrations involve breaks in whole chromosomes during the stage of growth that precedes replication. The pieces may then be rejoined (correctly or incorrectly), remain broken, or be lost. For each case, except a correct rejoin, the damage can be ascertained by visual examination under a microscope. Such observations are most commonly made in white blood cells because they are easy to obtain.

Part two of two parts.

Radiation-induced chromosomal aberrations have been recognized since the early 1950's. Inadvertent exposure of some workers to high doses of radiation has led to much information about dose-response relationships. In general, the number of radiation-induced aberrations that involves breakage of only one chromosome increases linearly with dose, whereas those that require breakage of two (to produce translocations, inversions, rings, and the more commonly studied dicentrics) increase approximately as the square of the dose. Chemical mutagens can produce similar effects, Sheldon Wolff of the University of California at San Francisco told the Rockefeller symposium, but "dosimetric problems become formidable. Neither the concentration of chemicals within cells nor the biological or chemical lifetimes are known with any certainty." Knowledge regarding diffusibility or active transport of chemicals into the cells in question is also limited.

The major problem with assessment of chromosome aberrations is getting statistically significant results for low dosages. Even after the relatively high radiation dose of 10 rads, for example, Wolff says that 300 cells would contain an average of less than one dicentric. "One would have to study an extremely large number of cells to observe a statistically significant increase." The number of aberrations produced by chemicals, some experiments suggest, will be smaller; if the exposure occurs over a long period, the number of aberrations will be further reduced by repair mechanisms.

Great care must be taken in assessing the significance of an increased number of abnormalities. "Even when one does see some aberrations," says Wolff, "it is difficult to say with any certainty that the aberrations were induced by the given exposure, because people are exposed to many other agents, such as medical and dental x-rays, caffeine, viruses, and other environmental pollutants, that can break chromosomes. This also raises difficulties in defining proper reference populations."

Wolff frequently cites the cautionary example of a radiation therapist who was exposed to excessive radiation when an x-ray machine failed to shut off while he was setting the machine for the next patient. "In 500 lymphocytes we found three dicentrics, which was certainly disconcerting. However, we also examined blood from the physician's partner, a diagnostic radiologist not exposed in the accident. The 'unexposed' partner had four dicentrics in 200 cells."

An increased number of chromosome aberrations believed to be associated with radiation exposure has been observed in atomic submarine refuelers in Britain. Relatively high doses of radiation, however, were required to produce observable numbers, the results were highly variable, and a worker whose sample showed an aberration on one occasion might have none later.

The health significance of such abnormalities is still questionable. Most aberrations that can be observed are actively selected against and are generally not passed on to daughter cells because they prevent cell division or because they lack a large number of necessary genes. "As a group, however," notes Wolff, "the exposed cells will have other [nonvisible] chromosomal changes, as well as point mutations. Therefore, the presence of aberrations can be used to signify a potential problem in the population, but quantifying the abnormalities in the seemingly normal cells is extremely difficult."

Support for this conclusion can be found in studies of Japanese who survived the atomic bombing of Hiroshima and Nagasaki. These studies show that, as the exposure increased, the number of chromosome aberrations and the incidence of cancer also increased. Adds Wolff: "The individuals with the aberrations, however, were not necessarily those who developed cancers."

The discovery of chromosomal aberra-

*A. D. Bloom, Ed., *Guidelines for Studies of Human Populations Exposed to Mutagenic and Reproductive Hazards* (March of Dimes Birth Defects Foundation, White Plains, N.Y., 1981); W. W. Lowrance, Ed., *Assessment of Health Effects at Chemical Disposal Sites* (The Rockefeller University, New York, 1981); "Research Needs for Evaluation of Health Effects of Toxic Chemical Waste Dumps," symposium sponsored by the National Institute of Environmental Health Sciences, held at Research Triangle Park, N.C., 27 to 28 October 1981.

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Mary Ann Carter/TIME Magazine

At greatest risk?

Workers who clean up dump sites may be the best subjects to study because they have the highest risk of exposure.

tions can thus introduce an ethical quandary. "What," asks Renate Kimbrough of the Centers for Disease Control, "do you tell the patient in whom such an observation has been made? Do you tell him that he'll get cancer in 20 years?" Scientists such as those investigating the Love Canal situation have been very insensitive about such problems, says Adeline Levine of the State University of New York at Buffalo. They have failed to recognize the impact of their findings on the morale and psychological well-being of the residents—to the point where many of the residents will no longer cooperate with investigations.

Another chromosomal abnormality that can potentially be used for assessing exposure is the sister chromatid exchange (SCE). These are exchanges of genetic material between complementary chromatids that have been made chemically different from one another (typically by treatment with bromodeoxyuridine) and thus differentially stainable. (Chromatids are identical chromosomes attached to the centromere during replication of DNA.) Such exchanges are readily visible under a microscope. They are also a more sensitive indicator of exposure, often showing significant increases in numbers at chemical doses a hundredth as large as those necessary to increase the yield of aberrations.

"The relation of SCE's to ill health, however, is even more tenuous than it is for chromosome aberrations," says Wolff. A March of Dimes panel chaired by Arthur D. Bloom of the Columbia University College of Physicians and Surgeons and Anthony V. Carrano of Lawrence Livermore National Laboratory notes that SCE's "are not, as far as we know, mutational events, in the strict sense. We are aware of no health consequences of SCE's per se." The main argument for use of SCE's for monitoring exposure to chemicals is the fact that many mutagens and carcinogens induce SCE's in cultured cells.

In attempts to monitor people exposed to suspect toxic chemicals, Wolff says, investigators have found only very few compounds that elevate the mean number of SCE's above that of reference populations. Those studies showing an increase in SCE's include laboratory workers carrying out hormone analyses and organic chemical research, nurses handling cytostatic compounds, and hospital workers exposed to the sterilant ethylene oxide. The elevated numbers of SCE's in these populations, however, were high only in comparison to nonsmoking controls: the smoking control population for the study of laboratory workers, for instance, had the same average number of SCE's as the population of smoking and nonsmoking exposed individuals. In conclusion, the Bloom-Carrano panel argues, "SCE studies in human populations are likely to prove to be a sensitive measure of exposure to chemical mutagens, but such studies are in their formative, or developmental stages, and must be interpreted with caution." Meanwhile, more work needs to be done on ways to identify mutations directly.

If it is not possible to monitor exposure directly, it may be necessary to infer exposure from studies of health effects. The most common approach to assessing exposure is the study of reproductive dysfunctions, including impotence, sterility, fetal loss, stillbirth, low birth weight, birth defects, and infant illness and mortality. Reproductive effects were a special focus of the March of Dimes meeting, and were considered by a panel chaired by Dorothy Warburton and Zena Stein of the Columbia University College of Physicians and Surgeons.

Impotence and sterility or infertility are generally not easily studied measures of reproductive dysfunction, says Warburton, except in the most unusual cases. Their incidence in the population is rather high and there are many confounding variables in coital and contraceptive practices and in the unrecognized or unreported loss of fetuses. Only when the health effects are striking, as was the case with kepone workers in Virginia and dibromochloropropane workers in California, do any of these become a worthwhile indicator.

Sperm studies are appealing, says Warburton, "because they offer the most direct look possible at the products of gametogenesis." Semen abnormalities are often analyzed, but the panel's consensus was that although these may represent indicators of exposure, their significance for reproductive function is largely unknown. Even among fertile men, sperm numbers and ejaculate volumes vary greatly, as may sperm motility. Single samples are insufficiently representative and, in populations under study, as many as half the men asked will refuse to provide specimens.

When the frequency of abnormalities is sufficiently unusual, sperm morphology (structure) can be useful, says Warburton, but "it is subject to great variation among observers, even with attempts to standardize classification schemes." It is known, moreover, that some men with abnormally low sperm counts or with large numbers of morphologically abnormal sperm are fertile, while other men with normal sperm are infertile. And finally, she says, there is little evidence for or against an association between sperm abnormalities and undesirable outcomes such as spontaneous abortions or birth defects.

The study of abnormal morphology is more sensitive than sperm counts though, argued Andrew Wyrobeck of the Lawrence Livermore National Laboratory at the Research Triangle Park symposium. A 25 percent change in the frequency of abnormalities can be detected with only 26 men, whereas a similar change in the number of sperm requires 200 men for detection. Studies of sperm abnormalities have been useful in certain

cases, he adds. When investigators suspected unusual exposures of workers involved in production of the pesticide carbaryl, for example, sperm counts were normal but there was a significant increase in the number of abnormalities. Wyrobeck further notes that there is normally very little difference in sperm counts or number of abnormalities over the course of an individual's lifetime, so that studies of individuals for long periods should provide a more effective indicator of exposure.

Spontaneous abortions, defined as termination of pregnancy before 28 weeks of gestation, may be a better indicator of reproductive dysfunction and exposure to toxic substances. "About 90 percent of chromosome aberrations [in germinal cells] are lost as the result of spontaneous abortions," says Warburton, "and about 50 percent of all pregnancies with malformations" are similarly lost. In normal populations, at least 15 percent of all recognized pregnancies are spontaneously lost, and the number in unrecognized pregnancies may be much higher. This high rate, like that of sperm abnormalities, makes spontaneous abortions a potentially sensitive indicator. A doubling in the rate of occurrence of a rare event, for example, is much more likely to occur by chance than a smaller increase in the rate of a more common event. Spontaneous abortions also have the advantage of providing a specimen that can be examined in various ways to provide information about the mechanism of loss, including chromosomal analysis and detailed morphological classification of defects.

There are also disadvantages. The only sources of available data are medical records and personal interviews. Medical records are insufficient because a large number of spontaneous abortions are not brought to medical attention, while interview data are subject to recall bias and to inaccuracies of self-diagnosis. Chromosomal and pathological examination of the abortus is also expensive.

Increases in the rate of spontaneous abortions have been reported among female anesthesiologists in England and the United States, in the wives of individuals who work with vinyl chloride, and in women near Alsea, Oregon, and at Love Canal exposed to the herbicide 2,4,5-T. The Warburton-Stein panel concluded, however, that each of these studies has serious deficiencies that make the data suspect. The studies of anesthesiol-

The Dump That Wasn't There

There is perhaps no better example of the difficulties in linking health effects to hazardous waste storage and dump sites than the so-called "ghost dump" in the Frayser neighborhood of Memphis, Tennessee. The situation that occurred at Frayser is also a good example of the perils of relying on residents' reporting of an increased incidence of health effects.

Frayser is a blue-collar residential neighborhood surrounding Steele Street in the northern suburbs of Memphis, not far from the Mississippi River. Memphis has a large chemical manufacturing industry, but none of the plants are in the immediate vicinity of Frayser. In August of 1976, Mrs. Evonda Pounds of Frayser called the Memphis and Shelby County Health Department to complain of rashes and other minor illnesses, arguing that she and her family were being poisoned by toxic chemicals in the environment. An investigation of her home and yard disclosed only trace concentrations of chlordane and other pesticides that are used in Memphis to control termites and mosquitoes.

Mrs. Pounds called again the next year, with the same complaints and the same results. And the next year. By the summer of 1979, other residents of the neighborhood had begun to complain of an increased incidence of rashes, headaches, urinary problems, heart disease, and cancer. One former health department employee claimed that he knew the location of a chemical waste dump in the area, says Charles Konigsberg, director of the Memphis and Shelby County Health Department. By the following April, he says, there was a "highly charged atmosphere," virtually identical to that at Love Canal; the citizens "panicked" and there was picketing and emotional meetings were held. There was even talk of evacuating the area. A local political activist pressed for immediate action in the area, and Representative Albert Gore, Jr. (D–Tenn.), held congressional hearings about the reputed dump, even though Frayser is not in his district.

Meanwhile, the U.S. Environmental Protection Agency (EPA) had begun analyzing air, water, and soil samples from the entire neighborhood. According to Jeff Harris of the Tennessee Department of Public Health, they found "absolutely nothing higher than background levels of pesticides." Working with EPA, epidemiologist Morton Korn of Johns Hopkins University went through old records covering the Frayser area, examined aerial photographs taken in the neighborhood since development began after World War II, and conducted other studies. Says Harris: "They found no obvious source of chemicals."

During the same period, the U.S. Centers for Disease Control (CDC) conducted a door-to-door survey of some 300 homes in the neighborhood asking about health effects, examined physicians' records, and so forth. This survey, according to Henry Falk of CDC, "did not produce any evidence of toxic illness or severe health effects." There was a slightly increased incidence of headaches and skin rashes, but the latter were of several different types and it is "unlikely that they were chemically caused." One man was found who had heavy metal poisoning, but he works in a foundry where the metal is present. One of Mrs. Pounds' children was found to have hives. CDC "took a long time" to publish their findings, says Konigsberg, and when they finally were released some 14 months ago, "nobody believed any agency."

About that same time, Memphis authorities became aware of the North Hollywood Street dump site some 3 to 4 miles away from the Frayser neighborhood—and separated by several physical barriers so that there is no chance of any chemicals from it having reached Frayser. The Hollywood site is a known waste storage area whose contents have been identified, and federal and local agencies shifted their investigations from Frayser to the more immediate hazard. "There are about half a dozen people in Frayser who still believe they have unusual health problems," says Konigsberg, but the uproar and the outrage have largely subsided. But the specter of this ghost dump will continue to haunt epidemiologists confronted with other self-reported increases in illness.—T.H.M.

ogists were based on questionnaires that could be biased if the frequency of abortions was higher among those individuals who replied than among those who did not. The vinyl chloride studies were based on husbands' recall of their wives' miscarriages, an approach that is considered unreliable. The Alsea studies were based on women hospitalized for spontaneous abortions, and there was no study of hospitalization rates among controls. Finally, the Love Canal study used inappropriate control data, collected 10 years previously from a different population.

Birth defects are much less common, occurring in only 2 to 3 percent of live births. This is the reproductive outcome that is most distressing to families and to society; it is also the most visible outcome, and thus the outcome most likely to be self-reported by the community as being increased. Anomalous fetal development, however, can have a great many causes, both genetic and physical, and can take a wide variety of physical manifestations. Until very recently, there have been no systematic records of malformations and little agreement about how to classify them.

Identification of a consistent pattern of malformations as a result of exposure to a particular agent is convincing evidence of a real effect, says Warburton, but it is also possible for the effects resulting from a single exposure to vary, depending on the time of exposure and the dose. In the case of chemical dump sites, where multiple agents are involved, the effects could be so varied that it will prove almost impossible to demonstrate any linkage. An increased incidence of birth defects, in fact, has not been convincingly demonstrated at any waste storage site. In the United States, the panel concludes, the necessary baseline information on spontaneous abortions and birth defects has not been developed on a sufficient scale, and should be vigorously pursued.

Birth weight of the offspring is another potential indicator of maternal health. About 7 percent of American infants weigh less than 2500 grams at birth. Low birth weight is the most important contributor to infant mortality, and is strongly related to congenital malformations, developmental delay, and chromosomal abnormalities. One of its chief advantages as a diagnostic tool is that birth weights are routinely recorded on birth certificates and hospital records in most parts of the world, and the data are not subject to biases of reporting.

For this reason, says Warburton, the reported increase in the number of low birth weight babies is the most convincing reproductive effect described at Love Canal and from the areas surrounding lead smelters in Sweden. But maternal age, race, alcohol consumption, and smoking habits are also risk factors for low birth weight. Gestational age should also be determined to distinguish between those infants born small due to prematurity and those born small due to poor fetal growth.

One type of assay for which the health effects are more predictable is neurotoxicological examination. Neurotoxic illness is generally diffuse; degeneration may occur in neurons, in their myelin sheaths, or in nerve junctions throughout the body. Neurotoxic impairment may thus produce a wide variety of clinical symptoms. Furthermore, says Herbert H. Schaumburg of the Albert Einstein College of Medicine, "subclinical impairment of the nervous system is prevalent" throughout the population without being noticed; further impairment is not recognized unless it is substantial.

Chemicals tend to exert their effects on the nervous system immediately rather than after a latent period. The manifestation of the symptoms may depend dramatically on the dose and the length of exposure. Massive doses of acrylamide, for example, produce convulsions, hallucinations, and encephalopathy, whereas mild exposure can cause peripheral nerve damage and blindness. One chemical, furthermore, can often potentiate the neurotoxic effect of a second.

Studies of potential neurotoxic effects have been conducted at many dump sites by a technique called nerve conductivity measurement. In this approach, a nerve (such as one of the long nerves of the leg) is stimulated at its upper end and the investigator measures the time lapse before the innervated muscle twitches. Presumably, a lengthening of the delay time represents nerve damage. The test is performed because it is easy, but Schaumburg does not think it is very discriminating: "I think it's an abominable test for most toxic neuropathy. By the time nerve conduction fails, you've got a badly damaged patient. Also, the test has to be done in the hands of a master, and it has to be very carefully standardized." He argues that some 90 percent of published studies in which the technique is used are suspect for one reason or another, and that many chemicals have been falsely labeled as neurotoxins because of its use.

He holds more hope for such newer techniques as evoked potential measurements (in which brain electrical activity is measured after other parts of the body have been stimulated), and for newer, noninvasive imaging procedures such as positron emission tomography and nuclear magnetic resonance spectrometry, which can monitor metabolic activity in the brain.

Echoing other investigators, Schaumburg says there is a "desperate need for neurodiagnostic technology" that can be applied by technicians to assess possible damage. There is also a need for animal tests that can identify potential neurotoxins. "Too often," he says, "the only test animal we have is man himself." Society has been lucky so far, he says, that there have not been any serious outbreaks of neurotoxicity at dump sites. There have been problems in industry, however, and it seems inevitable that new problems will be identified as the diagnostic technology improves.

Apart from certain neurotoxic effects—and obvious cases of outright poisoning—any health effects that result from hazardous waste storage or disposal sites will most likely be subtle and hard to detect. They will tend to be long-term and may remain asymptomatic and subclinical (undetected by routine physician or self-examination), possibly through long latent periods. Illnesses that do arise from chemical exposures, furthermore, can be expected usually to resemble or be identical to the conventional health problems in a community, such as cancer, miscarriage, and nerve damage—what Robert Goyer of the National Institute of Environmental Health Sciences terms "the most feared diseases."

One of the few areas on which agreement can be reached by nearly everyone is that such diseases can be detected only by long-term monitoring of populations at risk. So far, however, there are few such studies and limited funds to support them. One prototype study is being conducted by Harold Humphrey of the Michigan Department of Public Health. The department is monitoring 4000 Michigan residents who were exposed to polybrominated biphenyls in the early 1970's and 1600 Iowans who serve as a control population. The study is expected to continue for at least 15 years and should serve as a useful example of the effects of exposure to chemicals.

Another, more germane study is being initiated by Ruth Lilis of the Mt. Sinai School of Medicine in New York and Donald Henry of At-Sea Incineration, Inc., of Greenwich, Connecticut. They will monitor the health of workers aboard the ship that At-Sea will use to incinerate toxic wastes at sea. Many

investigators think this will provide the best approach to monitoring health effects, since individuals who dispose of toxic wastes are the most likely to have significant exposures. Lilis recommends that similar studies be initiated to monitor the health of workers who clean up dumps on land.

Refining these techniques for monitoring exposure and determining health effects, says James V. Neel of the University of Michigan, "is not an academic exercise but one of the two or three most important challenges to biomedicine in the next decade. Decisions entailing billions of dollars are involved. In this connection, I must once again chide many of my . . . colleagues for talking big but thinking small. We have spoken copiously of the possible risk to future generations of environmental contaminants, but, in my opinion, have thus far failed either to formulate or request funds to pursue proper epidemiologic studies. Hopefully, [these conferences] will be seen in retrospect as the occasions when [scientists] . . . first laid out to Government what had to be done in the nation's interest."

—THOMAS H. MAUGH II

The Hunter and the Starcloud

The great nebula in Orion is a tiny bright patch on an immense dark cloud; that cloud is the best place to study star formation

Orion, son of the sea god Poseidon, was a hunter of great beauty and strength. He was beloved of Artemis, goddess of the hunt. But he was carried off by the dawn goddess Eos, and Artemis slew him in a jealous rage. Later, he was placed in the sky.

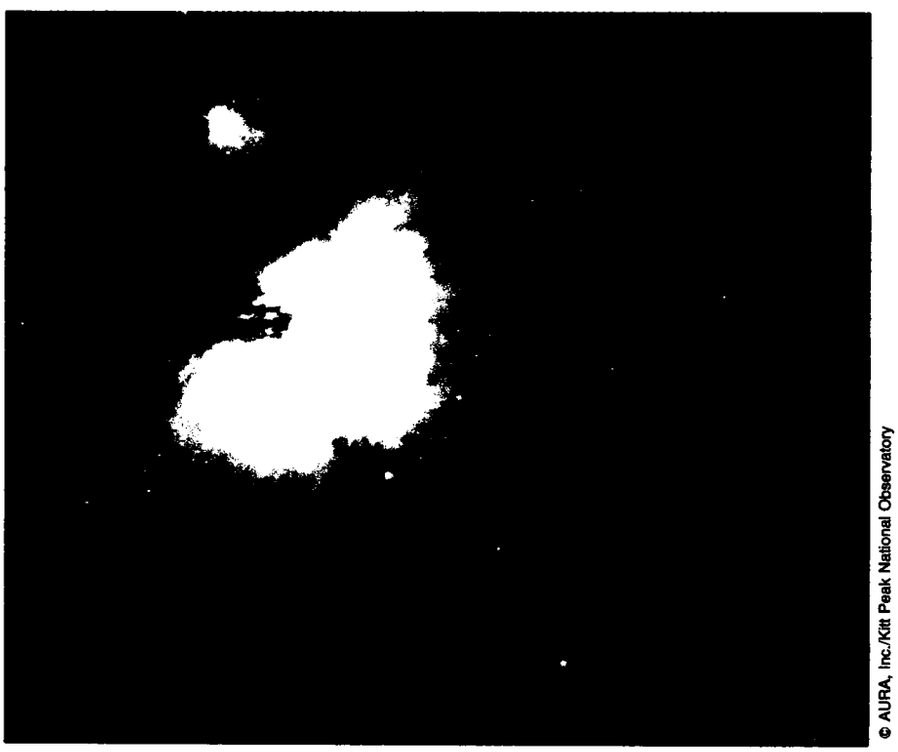
More recently, the legend has undergone a strange inversion: astronomers have come to regard Orion the hunter as a cosmic nursery. It is no accident that the constellation is so bright, they find. Most of the stars there, including all those in the hunter's belt and sword, are very hot and very young. They are blue-white stars of the O and B types, probably no more than a few million years old.*

Just behind those stars lie cold, dense masses of interstellar gas and dust, invisible against the blackness of space. These are the Orion molecular clouds. They sprawl across the hunter's midriff in a vast, broken complex hundreds of light-years across. In aggregate they contain enough material to make hundreds of thousands of stars like the sun. Stars are forming there now as denser clumps of material contract, turn their gravitational energy into heat, and finally ignite by nuclear fusion.

In the middle of Orion's sword, nestled against the face of the densest part of the cloud, lie the four stars of the Trapezium. They are the youngest of the visible stars in Orion. Their ultraviolet radiation is burning a blister in the cloud surface. The ionized atoms there respond by glowing brightly in visible light. This one tiny patch of the cloud complex is what we see as the Orion nebula.

The Orion molecular clouds are made mostly of hydrogen, with small admixtures of carbon, oxygen, nitrogen, and the solid grains of silicate dust. Their densest regions contain a million or more atoms per cubic centimeter. The surrounding interstellar medium contains about one atom per cubic centimeter. No one really understands how or why such giant molecular clouds form, but they are scattered along the spiral arms of the galaxy by the thousands. The Orion complex is neither the largest nor the most active at forming stars. It is, however, the closest to the earth. It lies in the lower fringes of the spiral disk, some 1600 light-years farther out from the center than the sun. Thus, it is by far the most accessible site for studying the poorly understood process of star formation.

Recently, some 200 astronomers interested in star formation gathered at New York University for a symposium on Orion.† The field has begun to develop quite rapidly in the last few years. Some of the data presented at the meeting were only a few months old.



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The great nebula in Orion

The young, hot stars of the Trapezium cluster, which energize the nebula, lie in the overexposed central region.

*Stars are classified according to their temperatures and spectral features. O stars are the hottest, M stars the coolest. Generations of astronomy students have learned to chant "*Oh *Be A* Fine *Girl,* Kiss *Me.*"

†"Symposium on the Orion Nebula to Honor Henry Draper," New York University, 4 and 5 December 1981. Draper (1837–1882) held appointments at New York University in the departments of natural history, philosophy, physiology, analytical chemistry, and chemistry and physics. On the night of 30 September 1880 he made the first successful photograph of a Nebula, the Orion Nebula.

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