Cell Cultures: Confused and Contaminated

Twenty-five years after the death of a Baltimore woman, Henrietta Lacks, from cervical cancer, cells from that lesion have quietly taken over tissue cultures in research laboratories all over the world. These "HeLa" cells once held only promise for research—they grow luxuriantly, are a substrate for growing viruses in the laboratory and are a steady source of experimental tissue. But now, as they silently contaminate and overgrow cultures that once contained dozens of other human tissues from twice that many donors, the promise of HeLa seems more of a menace. Two research groups, tracing Mrs. Lacks's *in vitro* immortality, have reported the extent and implications of the takeover for cell research.

Guy Vinson / U. of Calif. Naval Biomedical Research Laboratory

Nelson-Rees and Flandermeyer follow HeLa's tracks —chromosome markings specific to HeLa cells.

One team, Walter A. Nelson-Rees and Robert R. Flandermeyer of the University of California School of Public Health in Oakland, present in the Jan. 9 SCIENCE a list of 70 human cell lines once thought to be various types of human tissues but now known to have HeLa characteristics.

To set the scene, the list is a nearly illegible pastiche of abbreviations and acronyms—the shorthand names of cell lines such as "H.Ep.-2" and "NCTC 2544" and "HBT3" that make reports of cell research so impenetrable to the outside world. But the list means something very tangible to cell researchers. Work, sometimes done over several years on what was thought to be lung, kidney, amnion, prostate, skin, heart, breast, rectal and bladder tissues, was actually work done on HeLa cells. "In many instances, this invalidates the research," Nelson-Rees told SCIENCE NEWS, "and the researchers are going to have to acknowledge this in the literature."

Nelson-Rees has been tracing the spread of HeLa for several years. He and co-workers developed a set of chromosome staining and enzyme analysis techniques that will reveal the presence of HeLa characteristics. In 1974, they reported these techniques and the results of experiments on six HeLa-contaminated cell lines (SN: 6/15/74, p. 380). For the new list, Nelson-Rees and Flandermeyer not only analyzed and indicted 11 more cell lines but also compiled similar reports published by other researchers.

The new list indicts more than just cell lines, though. The investigators who originated these contaminated cultures have also been named, an action sure to be interpreted by some as unfriendly. "After the 1974 paper," Nelson-Rees explains, "some researchers analyzed cultures they had been using of the same type we 'fingered' and found them to be bonafide bladder carcinoma cells, or whatever. Therefore, the source of the contaminated cultures becomes an important piece of data, and I felt obligated to state from whence these cultures came and let the other shoe drop where it may." Although no one knows why HeLa cells grow so well, contamination is due, Nelson-Rees says, to sloppy laboratory technique, mislabeling of culture bottles and cross-culturing errors. "At this point," he says, half joking, "I'm going to go hide."

The second major report on HeLa contamination underscores this problem of investigator error. K.S. Lavappa, M.L. Macy and J.E. Shannon of the American Type Culture Collection (a major repository and distributor of tissue cultures in the United States) will report in an upcoming NATURE that 12 of their cell lines are also HeLa contaminated. ATCC has been sending these bogus cell lines out without knowing it, and is alerting researchers, through the NATURE paper, to the possibility that they are studying the wrong cells.

Of 63 refereed, characterized cell lines in the collection, Lavappa says, 28 are now HeLa suspects. They contain a rare enzyme, G6PD type A, which occurs in only 30 percent of Negroes and not in Caucasians. Henrietta Lacks, a Negro, had that enzyme, but most of the cell lines come from Caucasian donors. The ATCC team has checked 12 of 28 suspects so far, and all 12 were found to be HeLa contaminants. "But they did not get contaminated here," Lavappa says. "They must have been contaminated when submitted here by the investigators for preservation." Submissions are now being scrutinized more closely, he says.

Closer scrutiny of cell cultures in general is necessary in light of the HeLa findings, Nelson-Rees says. "The use of cell cultures for research has snowballed in recent years, but much of the lab technique is very sloppy." Both groups emphasize the necessity of checking and rechecking cultures for possible contamination, and would like to see this be a prerequisite for publication of research. □

Virus linked to diabetes

Diabetes is far more serious and widespread than most people realize. It is the third-ranking killer in the United States after heart disease and cancer; the incidence has increased by more than 50 percent in the past decade. There are many forms of the disease, such as acute onset juvenile, maturity onset and obesity onset. And all that scientists are sure about all of them is that the hormone insulin is lacking or malfunctioning.

However, one possible cause for diabetes has come to light in recent months. Several studies have suggested that the disease might be due to improper binding of insulin to receptor sites on the membranes of target cells, or that there may be too few of these receptors for efficient insulin binding (SN: 10/19/74, p. 248; 8/16/75, p. 110; 1/10/76, p. 23). Now another study provides some of the strongest evidence to date that a virus might help trigger one form of diabetes.

This study was conducted by Roger

Loria and Stanley Webb of the Medical College of Virginia at Richmond, and Sidney Kibrick and Gordon Madge at Boston University. They reported their findings at a meeting of the American Federation of Clinical Research in Boston this week.

Coxsackie B virus has been suspected of playing a role in diabetes. Loria and his colleagues have explored its role in mice predisposed to acute onset juvenile diabetes. Specifically, they used the virus to infect mice having a genetic predisposition toward diabetes, as well as to infect mice that did not. After infection, the genetically predisposed animals came down with diabetes. The virus could also be seen attacking their pancreases. The pancreas is the gland that makes insulin. In contrast, the nongenetically predisposed mice did not come down with diabetes.

"We are trying to see if this is, first of all, the only cause [of acute onset juvenile diabetes]," Loria told SCIENCE NEWS. "There may be many more causes. We'd like to know how the virus triggers the disease. Then we might think about how we can intervene."

One possible approach the investigators have suggested would be the manufacture of a coxsackie B vaccine. The vaccine could then be used to immunize children at special risk from acute onset juvenile diabetes. □

T-mycoplasmas and male infertility

During the past several years it has become apparent that a bizarre microorganism, a cross between a bacterium and a virus, may be responsible for certain cases of male infertility. In one study, for instance, Hakan Gnarpe and Jan Fridberg of the University of Uppsala isolated the microorganism, called the T-mycoplasma, from the semen of men with unexplained infertility. They suspected that the organism might have triggered the problem and gave the men antibiotics. Afterward the wives of 30 percent of the men became pregnant (SN: 9/22/73, p. 182).

Now two studies reported in the December FERTILITY AND STERILITY provide further evidence that T-mycoplasmas can trigger certain cases of reproductive failure among men. Research conducted by the investigators may also lead to a highly effective means of treating T-mycoplasma-caused infertility, as well as offer new approaches to birth control.

In the first study, Dana M. Fowlkes, Gerald B. Dooher and William M. O'Leary at Cornell University Medical College took samples of ejaculated sperm from infertile men and cultured them for the presence of T-mycoplasmas. Those ejaculates found to contain the microorganism were put in one group. Those found not to contain the microorganism were put in another group. The ejaculates from each group were then examined under the scanning electron microscope to see whether T-mycoplasmas actually adhered to the sperm in the ejaculates.

The sperm from the ejaculates containing the T-mycoplasmas could be seen to be coated with numerous spherical T-mycoplasmas, interlaced with fibrils, imparting a rough texture to the sperm.

O'Leary et al.

A sperm without T-mycoplasmas (top). A sperm coated with T-mycoplasmas (bottom).

The sperm had coiled tails, which may or may not be related to T-mycoplasma infection. In contrast, the sperm from ejaculates without the T-mycoplasmas were smooth. There was no evidence of T-mycoplasmas on them.

In the second study, Fowlkes, O'Leary and another Cornell scientist, John MacLeod, found that sperm from infertile men containing the T-mycoplasmas couldn't move as fast as sperm from infertile men without the microorganism. So they concluded that T-mycoplasmas may trigger infertility by decreasing the motility of sperm up the female vaginal tract. What's more, the binding of T-mycoplasmas may also allow them to slip past the normal microbial barrier of the female cervix.

Since their studies were submitted to FERTILITY AND STERILITY, the Cornell microbiologists and anatomists have also found that T-mycoplasmas don't have to be present to inhibit fertilization. Rather, they secrete a chemical that does it. "We are now working on isolating that material," O'Leary told SCIENCE NEWS. "Once we get that, then we can find out the actual biochemical lesion that causes all this to happen." Isolation of the chemical, he says, may also lead to a sure-fire therapy for T-mycoplasma-induced infertility. What's more, it may also offer a new approach to birth control. □

WMO: Limited SST fleet won't hurt

The World Meteorological Organization (WMO) released a study in Geneva last week predicting that the number of supersonic transports (SST's) now planned for commercial operation would not hurt earth's ozone layer, as many had feared. The latest in a long series of often conflicting reports on the subject was released just as the U.S. Secretary of Transportation, William T. Coleman Jr., was holding hearings on whether the British-French SST, the Concorde, should be forbidden to land in American airports because of its noise.

The WMO announcement calls for international agreement on the total level of permissible emissions, but says that with the limited fleets now planned—a total of 30 to 50 planes—no "significant" effect on the ozone layer should occur. Indeed, it said any effect would likely be indistinguishable from "natural variation."

However, according to the study, some damage might be done if much larger fleets were eventually put into service, or if the SST's were designed to fly higher than the present 10-mile altitude. The ozone layer lies mainly between 12 and 15 miles above the earth, and severe depletion would cause greater amounts of ultraviolet light to penetrate, probably leading to increased incidence of skin cancer. □