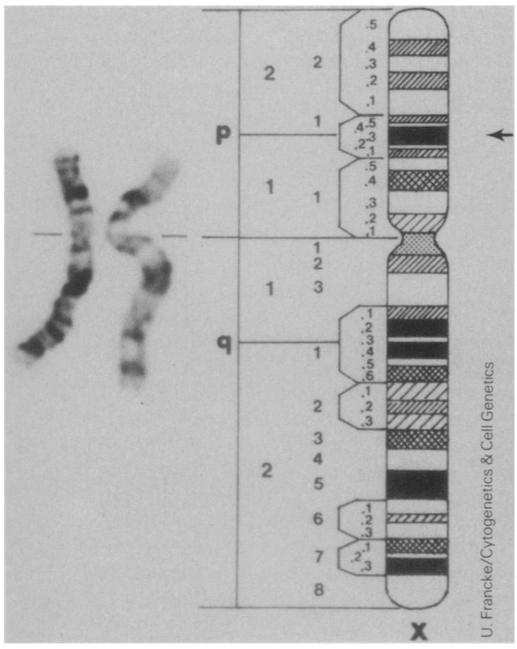
Duchenne Muscular Dystrophy: A Cure in Sight?

The protein and gene responsible for the disease may soon be identified, setting the stage for finding an effective treatment

By JOAN AREHART-TREICHEL

Duchenne muscular dystrophy, which afflicts thousands of American males, is the most common and devastating of all muscle-wasting diseases. Muscle deterioration starts in childhood and becomes progressively worse. Victims rarely survive beyond early adulthood. At present there is no cure for the disorder.

Although scientists have been trying to understand the causes of Duchenne's and to find an effective treatment for many years, they now appear to be on the brink of two achievements of unprecedented importance. One is identification of the protein responsible for the disease. The other is isolation of the gene on the X chromosome that codes for the culprit protein, the gene affected sons inherit from their unaffected mothers. "Identification of the protein and gene would be the greatest research coup since the disease was first described in 1861," declares Melvin Moss, director of research for the Muscular Dystrophy Association in New York City. "The reason is that once scientists determine the basic cause of the disease, rational approaches to therapy could be undertaken."



Two human X chromosomes photographed under the microscope with explanatory diagram. "P" refers to the upper half of each X chromosome, "q" to the lower half. The arrow points to band 21, the place on the upper half of the X chromosome where the Duchenne muscular dystrophy gene appears to be. The P21 band is further broken down into five sub-bands; scientists are not yet sure on which of the sub-bands the gene is located.

The research that appears to be bringing scientists close to identification of the Duchenne protein comes from Edward Rosenmann and his biochemistry team at the University of Manitoba in Canada. As they reported in the Aug. 5 NATURE and at the International Congress on Neuromuscular Diseases in Marseilles, France, in September, they have found that a particular protein is missing in cells from Duchenne patients. This is the first time that a *protein* abnormality has been noted in cells from Duchenne patients. It's conceivable that the missing protein is the one responsible for Duchenne.

A complex scenario led Rosenmann and his team to the missing protein. Although the major pathological changes in Duchenne occur in skeletal muscle, minor ones have also been detected in other parts of the body, including connective tissue cells called fibroblasts. These quirks are probably all caused by the same gene and protein because all cells in a Duchenne patient's body contain essentially the same genetic material. Thus, if a protein defect could be determined in a non-muscle cell from a Duchenne patient, it might well be the same flaw that makes muscle cells in the patient dystrophic. Fibroblasts are easier to obtain than muscle cells are, and they are easier to study. Rosenmann and his colleagues decided to look for the responsible protein there.

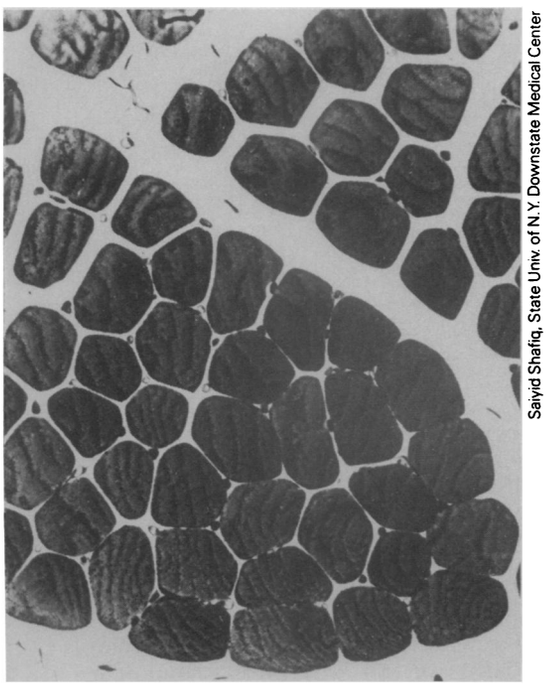
They took fibroblasts from Duchenne patients and from healthy subjects and attempted to see whether there was any difference in protein composition. They found one—a protein of about 56,000 daltons in molecular weight was present in cells from healthy subjects but not in cells from patients. "This is the first time," Rosenmann told SCIENCE NEWS, "that differences between normal and dystrophic cells can be attributed to one protein." He cautions, though, that "for the moment we cannot really say this is the primary defect responsible for the dystrophic condition."

Still, he and his colleagues hope it is and will try to collect more evidence to prove it. It's conceivable that the missing protein is an enzyme because enzyme deficiencies are now known to underlie three inherited muscle diseases other than Duchenne.

The imminent prospect of isolating the gene that makes the culprit Duchenne protein comes from a number of genetic teams working independently yet cooperatively. What is remarkable about their efforts is that they are confident that they will be able to isolate the gene and even agree on when they will do so. They estimate that they will have a genetic marker for the gene — that is, genetic material close to the gene—in hand within the next 18 months and the gene itself located within the next four or five years. The reasons, explains Uta Francke of Yale University School of Medicine, "are that all the tools are available, there is enough interest, and there are enough good people working on the project."

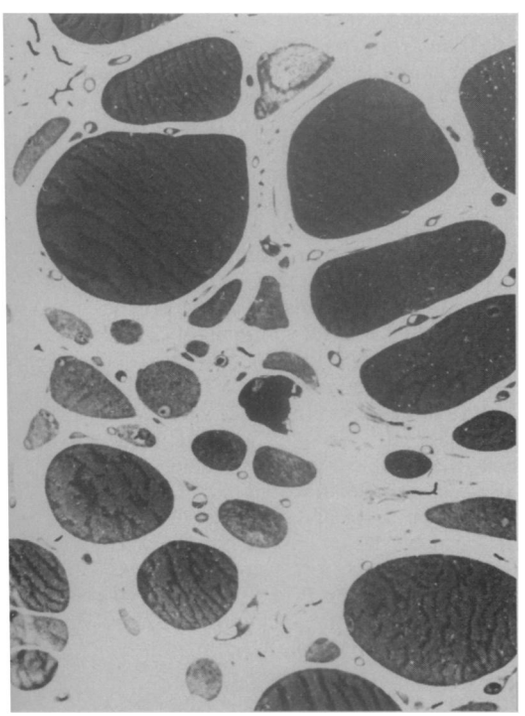
Although there are variations in their tactics, their thrust takes a basic form. First, blood cells are taken from the normal, healthy population. DNA is extracted from the cells, and enzyme splicing is used to generate large numbers of specific DNA fragments. Those DNA pieces on the short arm of the X chromosome are the ones of interest because microscopic evidence obtained during the past several years suggests that the Duchenne gene is in the middle of the short arm.

Then the geneticists look for variations in the pieces. If such variations exist, the geneticists take genetic material from the blood cells of Duchenne patients and their families and analyze it to see if there is any particular variation that is always present in Duchenne patients and Duchenne carriers. (Preliminary evidence that this is the case, in fact, was reported in the Nov. 4 NATURE by Robert Williamson and colleagues at the University of London.) Such a gene variation would serve as a genetic marker for Duchenne muscular dystrophy. In



Sayid Shafiq, State Univ. of N.Y. Downstate Medical Center

Normal muscle cells.



Duchenne muscle cells, being replaced by connective tissue.

other words, because genes located close together on a chromosome tend to be inherited together, the presence of a particular DNA fragment version in Duchenne patients and carriers would mean that the version is located close to the Duchenne gene and inherited with it, thus serving as a genetic marker for the gene.

The geneticists will then use the marker to isolate the Duchenne gene itself. First, they will try to see if there are any differences in the marker between Duchenne patients and healthy siblings. If not, the marker would *not* be the gene. Efforts will then be made to see whether DNA pieces flanking the marker differ between Duchenne patients and healthy siblings. If a fragment is found to be peculiar to the patients but not to their healthy siblings, it will most likely be the Duchenne gene.

Isolation of the gene responsible for the disease could probably then swiftly lead to identification of the protein coded by the gene. For instance, messenger RNA could be taken from Duchenne muscle cells and analyzed to see whether any particular stretch of it matches up with the Duchenne gene. If so, the piece would be the mRNA that codes for the gene, and it could be translated to see what kind of protein it makes.

And once scientists have the culprit protein in hand, it should lead them to the basic cause of the disease and put them in a position to find a cure. How long will that take? "It's hard to predict," admits one of the geneticists in search of the Duchenne gene, James Gusella of Massachusetts General Hospital in Boston. "But obviously I think this is the way to get at it."

Meanwhile, a drug that counters Duchenne to at least some extent may emerge. One particularly promising candidate at the moment is a drug that can keep calcium from entering muscle cells.

The promise of calcium blockers stems from important insights into the pathology of Duchenne-afflicted muscle that have been made over the past quarter-century. These insights suggest that, in Duchenne victims, calcium from the bloodstream passes through leaks in the surface membranes of muscle cells in large amounts, and once this excess calcium is in the membranes it somehow sets the stage for muscle wasting. These findings likewise imply that a drug that blocks the passage of calcium into muscle cells might benefit Duchenne patients. Or as Neil Lewis, coordinator for the MDA's Task Force on Drug Development, puts it, "Most people don't think such a drug will be a cure, but they are hoping it might have some major impact in controlling the progression of the disease."

Clinical trials to test calcium blockers in Duchenne patients are already underway. The MDA is sponsoring one at four American medical centers to test the calcium blocker nifedipine, and one at one American medical center to test the calcium blocker diltiazem. Both drugs were recently approved by the Food and Drug Administration to treat heart disease. Still other calcium blockers are being tried on Duchenne patients in other countries.

Other drugs that look as if they might have some impact on Duchenne are those that block proteases (enzymes that chew up proteins). The rationale for trying protease blockers, like that for trying calcium blockers, has emerged from various insights into Duchenne muscle cells. Specifically, once calcium moves into dystrophic muscle cells, it seems to activate proteases inside the cells. The proteases then seem to chew up proteins, causing the cells to ultimately die.

Protease inhibitors have already been found to delay muscular dystrophy in chickens (SN: 5/6/78, p. 298) and to counter muscle degeneration in mice (SN: 1/16/82, p. 39). Preliminary clinical trials testing the inhibitors have been conducted in Japan, and the MDA is eagerly awaiting results from them. If the association decides to fund a protease inhibitor trial, Alfred Stracher of the State University of New York Downstate Medical Center in Brooklyn, N.Y., and colleagues are ready to run it. They have obtained FDA permission to test the protease inhibitor leupeptin (SN: 1/16/82, p. 39).

Lewis cautions, though, that because the Japanese trials have used such small patient populations, it will be difficult interpreting results from them. Also, he says, "because calcium entry into the dystrophic cell appears to occur prior to activation of the proteases, it would appear that drugs blocking calcium entry would presumably be more effective earlier in the disease than would drugs that block proteases." □