

Medical Alchemy: Muscle Turned to Bone

In a modern twist on the alchemists' dream, a surgeon and a cell biologist have transformed shapeless muscle into hard, well-formed bone. The pair have just reported molding rat muscle into tiny leg and jaw bones. Their ultimate goal, however, is far loftier: production of genuine bone replacements for people injured in car accidents or crippled by disease.

"The beauty of our experiment is that the muscle melts away and everything turns into bone," says plastic surgeon Roger K. Khouri of the Washington University School of Medicine in St. Louis. Together with Hari Reddi of Johns Hopkins University in Baltimore, he has literally remolded soft tissue into firm bone.

The researchers isolated their starting material from the thighs of 23 rats. They cut into a piece of muscle and lifted out a flap of this tissue. They left it connected to an artery and a vein so that the muscle would continue to receive nourishment throughout the experiment.

In 18 animals, the researchers injected the muscle flap with osteogenin, a naturally occurring protein in bone that scientists believe may play an important role in the development of the fetal skeleton. Khouri's team then inserted the muscle flaps from the 18 osteogenin-treated rats into small rubber molds packed with pulverized bone. This pulverized bone probably contains additional growth factors, Khouri says. The five untreated flaps were packed into molds without the pulverized bone. Finally, the mold of muscle was surgically inserted into a pouch within each rat's abdomen.

After 10 days, the researchers reopened the pouches and withdrew the molds. All muscle treated with osteogenin had undergone a complete transformation to bone, Khouri and Reddi report in the Oct. 9 *JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION*. Depending on the mold, this tissue resembled long bones of the leg, the jaw's mandible or the ball-like tip of the femur (thigh bone). Despite a similar incubation, the five flaps without osteogenin retained the soft, formless texture and cell types characteristic of ordinary muscle.

"A lot of growth factors like osteogenin may actually be very important in terms of fetal development," Khouri says. In fact, this experiment was designed to mimic a process that goes on in the womb, when the tiny embryo's shapeless tissue starts to form a skeleton, Reddi explains. Although researchers believe osteogenin directs that early event, the body may use this protein later in life to heal broken bones, he says.

The researchers don't know whether the newly fashioned bones are as strong as normal ones. For instance, Khouri says the femur they created appears spongy, and perhaps weaker than normal. While scientists know that such spongy bone can become stronger with exercise, researchers must do additional studies before they can evaluate whether such manufactured hard tissue offers a suitable replacement for the leg bones, which must support a lot of weight, he says.

If scientists can identify and mass produce such substances as osteogenin, surgeons might one day garner the ability to build replacements for other types of damaged tissues, including heart valves, comments surgeon William Shaw of the University of California, Los Angeles. Indeed, he adds, "This is the beginning of a whole different approach to surgery."

Shaw speculates, for example, that bioengineers may one day use osteogenin to

fashion the ball-like top of the thigh bone, an advance that could provide longer-lasting relief to people suffering from osteoarthritis, a degenerative disease affecting the hip. People with this condition often suffer excruciating pain from years of stress and inflammation that damage the hip's ball and socket. To fix the problem, surgeons usually replace the damaged femur tip with a metal ball-shaped prosthesis. However, a femur tip fashioned from the person's own muscle should last longer and induce fewer infections, Shaw says.

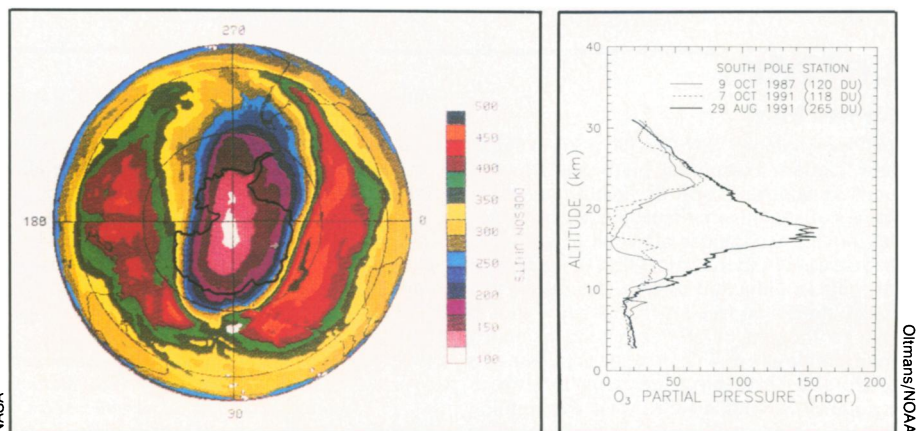
"It sounds like science fiction," acknowledges Khouri. Though cautioning that their data come only from rats, he says the team might extend the bone remodeling experiments to human tissues within a few years. Whether those studies provide humans with natural replacements for worn out parts remains to be seen. Clearly, Shaw says, the work has a long way to go. — K.A. Fackelmann

Antarctic ozone hole sinks to a record low

Atmospheric pollutants have again left their mark in the skies over Antarctica as this year's ozone hole reached maturity last week. The atmospheric concentration of ozone dwindled to its lowest recorded level, according to satellite measurements. The dramatic ozone depletion marks the third year in a row that a severe ozone hole has developed over Antarctica, in contrast to the 1980s when major holes formed only in alternate years.

"Three years in a row of low ozone leads one to wonder that maybe most years will be low in the future," says Charles H. Jackman of NASA's Goddard Space Flight Center in Greenbelt, Md.

The term ozone hole refers to a marked reduction in the number of stratospheric ozone molecules. Such a hole has formed over Antarctica each September since the late 1970s. Energized by the first faint rays of sunlight in the austral spring, chlorine chemicals in the atmosphere destroy



Data from the Nimbus-7 satellite show the ozone hole over the Antarctic continent (outlined in black). The oblong blue ring marks the "edge" of the hole, while white patches over Antarctica represent areas of most severe ozone loss. White blotches over other parts of globe denote regions of missing data. Graph shows vertical measurements of ozone values taken by balloon-borne instruments. The thick solid line demonstrates normal ozone values, while the thin solid line shows the 1987 ozone hole. The hatched line from October 7, 1991, shows the main region of ozone loss from about 12 to 23 kilometers in altitude. Also visible this year is an upper region of ozone loss from 27 to 29 km in altitude.

ozone in a layer between 12 and 23 kilometers in altitude.

Although winds from the north replenish the lost Antarctic ozone in November and December, the ozone hole's growth each year may be contributing to a general thinning of the global ozone layer, which satellite and ground-based instruments have detected over the past decade. Ozone blocks out harmful ultraviolet radiation from the sun, and scientists say the weakening of this shield will increase the risk of skin cancer in humans and will harm animals and plants.

On Oct. 6, the concentration of ozone over parts of Antarctica dropped to 110 Dobson units, beating a record of 121 Dobson units, set in 1987, says NASA's Arlin Krueger. The measurements were made by the Total Ozone Mapping Spectrometer on board the Nimbus-7 satellite. Normally, ozone levels for this time of year would be about 225 Dobson units, Krueger says.

This year's ozone depletion signals a clear departure from a pattern established in the 1980s, when severe ozone holes developed only on odd-numbered years. Weaker ozone holes appeared on the alternate years because the polar atmosphere turned more turbulent than normal, allowing warm winds from the north to penetrate into the polar region. In contrast, severe ozone loss occurred when the Antarctic atmosphere remained stable and cold during winter. After two strong ozone holes in 1989 and 1990, some scientists thought 1991 might bring a weaker hole, but the atmosphere remained cold and stable.

Jackman speculates that the intense ozone loss of the last few years may account for the development of a major depletion this year. Such a feedback could occur because ozone destruction helps maintain the cold polar temperatures needed to form the ozone hole. Normally, when sunlight returns to Antarctica in the springtime, ozone in the polar stratosphere absorbs the light and warms the upper atmosphere. But when half the ozone has disappeared from the polar region, the stratosphere stays much colder than normal even into the next year — a condition that could lead to severe ozone loss year after year, Jackman says.

"I don't think it's a good indication for ozone in general," he adds.

Balloon measurements made at the South Pole reveal an unusual character in this year's ozone depletion, one not seen in previous years, says Samuel J. Oltmans of the National Oceanic and Atmospheric Administration in Boulder, Colo. Along with the familiar pattern of loss in the lower stratosphere, ozone also disappeared from a layer between 27 and 29 km in altitude. The significance of the upper thinning remains unclear, says Oltmans, who is now attempting to determine when it first appeared. — R. Monastersky

Sex abuse: Direct approach may aid recall

A new study suggests that sexually abused children may best remember whether their genital area had been touched by an adult if asked directly about such experiences with the help of an anatomically detailed doll. Moreover, children show considerable resistance to the types of misleading questions that may inadvertently crop up in sexual abuse investigations, maintain psychologist Karen J. Saywitz of Harbor-University of California, Los Angeles Medical Center in Torrance and her colleagues.

Their study, published in the October *JOURNAL OF CONSULTING AND CLINICAL PSYCHOLOGY*, represents a rare attempt to develop guidelines for obtaining information about sexual abuse from children, based on an analysis of youngsters' memories for a real-life situation — genital touching by a pediatrician during a routine medical exam.

Such instances differ considerably from actual cases of child sexual abuse, caution Saywitz and her co-workers. In addition, questions about sexual abuse usually occur within repeated clinical interviews and stressful cross-examinations in the courtroom. Nevertheless, these data suggest that mental health clinicians and lawyers should not resort to direct questions about specific forms of abuse only as a last measure in sex abuse cases involving children, they contend.

"Our results indicate that although there is a risk of increased [memory] error with doll-aided direct questions, there is an even greater risk that not asking about [genital] touch leaves the majority of such touch unreported," the researchers conclude.

In actual sexual abuse evaluations, police officers or lawyers often first ask children general questions about what happened, then give them anatomically detailed dolls or other props to act out the incident, and finally ask specific — and sometimes misleading — questions about genital touching and sexual behavior.

Saywitz's group studied 72 girls, half of them 5 years old and half age 7. In each age group, half the girls received a standard medical check-up conducted by a female pediatrician with their mothers present, including a vaginal and anal examination. The remaining girls underwent the same procedure without vaginal or anal touching. Either one week or one month later, experimenters asked half of each group to report what the physician did to them through free recall of the visit, with demonstrations using an anatomically detailed doll, and in response to direct and misleading questions.

Only eight of 36 children who underwent the genital exam remembered vaginal touching through free recall and six through doll demonstrations; four children correctly reported anal touch in free

recall and in demonstrations. No children in the untouched group recalled or demonstrated that genital touch had occurred.

In contrast, 31 girls who received a genital exam disclosed vaginal touch in response to an interviewer's direct question accompanied by pointing to the doll's vaginal area. When asked direct questions, 25 girls in the same group reported anal touch. One child in the other group falsely reported vaginal touch under direct questioning, and two youngsters falsely reported anal touch.

Older children displayed better memory after one week than their younger counterparts, but this difference disappeared after one month.

Children made few errors in response to misleading questions (such as "How many times did the doctor kiss you?"), but younger children showed a greater tendency to err when misled.

Children's reports of when the exam occurred and the pediatrician's age and height achieved greater accuracy when the interviewer used age-appropriate questions, such as "Is the doctor old enough to be a mommy?" — B. Bower

Nuclear waste delayed

The Department of Energy last week temporarily delayed shipments to the nation's first permanent repository for nuclear waste, thus avoiding an immediate showdown with the state of New Mexico, where the underground facility is located.

Energy secretary James D. Watkins had announced in early October that the \$1 billion facility was ready to open and informed the state that initial shipments could arrive by October 10 (SN: 10/12/91, p.228). The day before that deadline, New Mexico filed suit in federal court, seeking to temporarily block shipments until Congress could pass a pending bill that specifies limits on the amount of waste the repository can store during a six-year testing phase. As New Mexico filed its suit, DOE decided to delay the initial shipments until a federal judge can rule on the state's case. Judge John G. Penn of the U.S. District Court for the District of Columbia scheduled a hearing for Nov. 15.

Located 653 meters below the desert surface near Carlsbad, the repository will hold radioactively contaminated waste, generated during the production of nuclear weapons. After completing the testing phase, the Energy Department must show the facility meets federal environmental regulations before filling the repository with 850,000 55-gallon drums of waste. □