JOHN HAYES

Their lives in his hands: athletes' reputations can be shattered if Don Catlin finds a banned drug in a sample.

No dope

Don Catlin's lab has struck a major blow against drug abuse in athletics, by developing a test for a shadowy 'designer steroid'. Jonathan Knight visits the scientists who are striving to keep sport clean.

When you arrive at the Olympic Analytical Laboratory in Los Angeles, it's clear that this isn't an operation that goes out of its way to make visitors feel welcome. Nestled among car-body shops, its narrow façade shoulders up against chain-link fences topped with razor wire. The mirrored-glass front door of this drug-testing lab is locked, and will only be opened if you are meant to be there. Numerous television crews have been turned away in recent weeks, and a sign inside the door reminds staff not to admit an athlete who filed a complaint against the lab after it found steroids in her urine.

Although the fences actually belong to the neighbouring car-repair yards, this defensive appearance is fitting for a lab that is at war with the suppliers of performance-enhancing drugs. After a summer of intense work on the residue from an anonymously supplied syringe, the lab rocked the world of sport last month by revealing that an undisclosed number of athletes had tested positive for a designer steroid, known as tetrahydrogestrinone or THG, which had evaded standard tests (see *Nature* **425,** 752; 2003).

Keeping things under wraps is an essential part of the game, says lab director Don Catlin. Indeed, most of the lab's employees were in the dark about THG until after the news became public. "If what we were working on had gotten out, people would have stopped using it before we had a test," Catlin says.

Drug testers and drug-using athletes have been locked in battle for more than three decades. When the International Olympic Committee (IOC) ran its first drug tests during the 1968 games in Mexico City, no one was found using anything other than alcohol. But heavier testing in Munich in 1972 caught seven athletes using banned drugs. Since then, testers and dopers have been engaged in an arms race — with the users of illicit drugs generally being perceived to have the upper hand.

An unlikely hero

Catlin did not expect to become involved in this struggle. In 1982, he was an established endocrinologist at the University of California, Los Angeles (UCLA), when the IOC came to ask if he would run the testing for the 1984 Los Angeles games. "They showed me a list of banned substances," Catlin recalls, "and I said, 'I don't think we can do this'." But when the IOC made it clear that it was offering to greatly expand his lab and hire the staff he would need, he rose to the challenge.

Today Catlin's lab, which is still part of UCLA, has three dozen employees. Its biggest customers, apart from the US Anti-Doping Agency (USADA), are the National Collegiate Athletic Association and the National Football League. Most of the work involves the routine processing of number-coded urine samples that arrive by courier almost daily. These can be analysed for several dozen hormones, stimulants, diuretics and other banned substances, depending on what the customer asks for. Most of the tests involve a combination of gas chromatography and mass spectrometry. The desktop machines that run these procedures occupy almost every available surface in the lab.

Besides this routine, there is a continuous effort to improve the tests. In the EPO lab, where technicians run antibody tests to detect the hormone erythropoietin, which boosts red-blood-cell counts so that more oxygen gets to the muscles, group leader Andreas Breidbach is looking for ways to see further back in time. Right now, evidence of EPO use is hard to find after about a week. But the benefits last longer. Knowing this, many athletes stop doping ahead of an event. Breidbach hopes to extend the window of detection by tweaking each step of the test to improve its sensitivity. "We are always improving because the athletes are always improving," he says.

One of the lab's research achievements is a method for spotting testosterone abuse. Unlike synthetic steroids, testosterone and its breakdown products are naturally present in the urine of dopers and non-dopers, whether they are male or female. The standard test for testosterone doping looks for an elevated ratio of the hormone relative to its cousin epitestosterone. These are normally present at equal levels in the urine, and the IOC has set a ratio of 6:1 as the cut-off for declaring a positive test. This is bad news for the rare athlete with a naturally elevated ratio. And the test is not hard to beat: athletes can, for instance, take epitestosterone as well as testosterone.

Catlin's group hit on the idea of using carbon isotope ratios to distinguish between natural testosterone and its lab-made counterpart. Synthetic testosterone contains less of the heavy isotope carbon-13 compared with the natural compound. This is because the hormone's manufacturers begin their synthesis with an extract from yams, which happens to contain a compound with the same four-ring structure as steroids. Plants incorporate carbon-13 — present at low levels in the atmosphere — much less efficiently than do animals. Although the isotope test is expensive and not always used, it has made a difference. Once athletes became aware of the test in the late 1990s, many stopped using testosterone, Catlin says.

Peak performance

But the battle continues. Just how far ahead are drug-using athletes and their suppliers? No one knows for sure, although part of the answer may lie in a slim binder tucked away on a shelf in the spectral analysis room at Catlin's lab.

In this room, Yuliya Kucherova pores over gas-chromatography traces and mass spectrograms, looking for the signatures of steroids. In some cases, the steroid shows up as a distinct peak by gas chromatography, which separates compounds based on their volatility. In others, confirmation by mass spectrometry is essential. This involves breaking molecules into fragments and determining their molecular weights.

Kucherova, after reading dozens of spectra a day for years, has no trouble spotting banned compounds. But occasionally a new peak shows up that doesn't correspond to any known drug, prescription or otherwise. Is it a new synthetic steroid? A Chinese herb? A rare prescription medication? These mystery peaks wind up in the special binder, and several dozen are awaiting further investigation.

Once in a while, Catlin and his colleagues get lucky. Kucherova last year spotted a peak she had actually seen previously. It was the synthetic steroid norbolethone, first made in 1966 as a possible treatment for short stature. But it proved too toxic and was scrapped by its maker, the pharmaceutical company Wyeth. It has not been made commercially since.

Although only one athlete tested positive for norbolethone, that result was a turning point for the lab. It helped Catlin to convince the sporting authorities that the underground market for steroids was being served by clandestine chemists and manufacturers. He made the case that to keep ahead of the game, the testers needed to work in secret on what the next underground drug might be. Earlier this year, the USADA awarded Catlin's lab a grant to probe such questions.

The group was preparing to launch into this work when the anonymously posted syringe came through the door. A team of three, which rapidly rose in number to eight, was immediately assigned to the task of identifying the residue inside. People worked late, sometimes all night. Details of the investigation were shared internally on a 'need-to-know' basis, but the sense of urgency and excitement was pervasive. "We've never had a project of this magnitude," enthuses Michael Sekera, the lab's scientific director. "It was an amazing experience."

It was clear right away that normal tests would never have spotted THG. Before analysis, samples are chemically treated to make steroids show up as single sharp peaks in the gas chromatograph. But this process sees THG produce 25 little peaks that don't look like a steroid at all.

Urine trouble

The researchers turned to mass spectrometry to get a clean signature. Then they worked backwards to guess at the compound's structure. They confirmed their hunch by making the compound from scratch and showing that it produced the same signature pattern. Then they found a primate laboratory that was willing to give THG to a baboon, so that they could have some idea how it would come out in the urine. Catlin and his colleagues devised a new test, and began running it on athletes' urine samples. The whole process took two months.

The story isn't over, Catlin says, as he anticipates that there may be legal challenges. These have become more frequent as the stakes in sports have risen. When Catlin first started in the drug-testing business, athletes often reacted to being caught with resignation, even contrition. "You caught me doc, I messed up," Catlin recalls one athlete saying. Now, athletes' lawyers send chemists into the lab in the hope of finding flaws in its testing procedures.

So Catlin has to be very careful. He won't discuss the results of the THG tests as long as the federal investigation into the scandal continues. Not that he knows the names of the accused athletes. All samples are coded before they arrive. Even an athlete's attorneys, who are entitled to observe the testing, say only which number they represent, and not which athlete.

In any case, Catlin and Sekera say that they are more interested in where to go from here. To keep ahead of the people making drugs you have to start making them yourself, they argue. You need to design them, find out how they break down in the body, and develop a test for them.

Whether the lab will get the funds to continue with this approach is a matter for the sports authorities to decide. The bulk of the USADA grant was consumed in figuring out how to test for THG. But if Catlin and his colleagues do resume this work, don't expect them to breathe a word of what they're up to. "We are very good at keeping things to ourselves," Catlin says. ■

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By the numbers: identified only by a code, rows of athletes' urine samples are screened for a wide range of banned substances.

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