Performance-Enhancing Drugs in Sports: How Chemists Catch Users

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What do the following prominent athletes have in common: Floyd Landis, Mark McGwire, Marion Jones, Ben Johnson, and Lyle Alzado? All have been suspected of using performanceenhancing drugs (PEDs) in recent decades and have eventually confessed. Over this period, major doping investigations have included the BALCO (Bay Area Laboratory Co-Operative) case, which involved doping in various sports instigated by a company in the San Francisco area, and the Mitchell Report, which describes PED use (particularly steroids) in Major League Baseball (MLB). Moreover, the Tour de France has been wracked by doping allegations for decades, causing teams to lose sponsors recently and struggle to maintain fan support, even in cyclingcrazy Europe. All of the sports headlines generated by these incidents have a chemical "back story" that is rich in relevant examples for undergraduate chemistry and biochemistry courses, and of particular interest to students pursuing careers in analytical or forensic chemistry, medicine, or pharmacy. These examples also provide an opportunity to teach chemistry with a social context, because the limitations to testing for PEDs in sports are not only technical (detection limits and selectivity), but also ethical (how invasive should testing be?) and economic (can we afford truly effective testing?).

The use of PEDs has been documented since early sports competitions between ancient Greeks and Romans (1). At present, organizations such as the World Anti-Doping Agency (WADA), National Football League (NFL), MLB, and the National Collegiate Athletic Association (NCAA) promulgate prohibited lists of doping substances and methods. The WADA list, which is the most extensive, is reviewed annually and can be found at the WADA Web site (2). The prohibited substances can give athletes an unfair competitive advantage or harm their health, and their use is contrary to the spirit of sport.

We have chosen to cover a variety of doping substances that provide timely and appropriate examples of chemical and biochemical concepts and methods. Most students have at least a passing interest in sports, and student knowledge of PED use by prominent athletes is often significant. As a consequence, students are quite responsive to pedagogical examples from the arena of PED testing in sports. This material has been used in a course entitled "Chemistry and Athletic Performance", which features the "cat-and-mouse game" between enablers of PED use among athletes and testers who try to deter such use. The course was developed with the assistance of Don Catlin, M.D., one of the "fathers" of sports drug testing, and Caroline Hatton at the UCLA

Olympic Analytical Laboratory and Anti-Doping Research, Inc. A description of the course can be found on the Web (3).

Stimulants

Athletes have used stimulants, such as caffeine, strychnine, and cocaine since the 19th century (4). The 2010 WADA list prohibits all stimulants in competition and names about 60 examples. The most notorious is amphetamine (Figure 1), which stimulates the central nervous system by releasing neurotransmitters, such as dopamine, into the synapse between neurons. As a result, amphetamine combats fatigue (5). Stimulant abuse puts athletes at risk for heart rhythm anomalies and for injury to themselves and their competitors, among other things.

Amphetamine use has been rumored for decades to be widespread in baseball. MLB first tested for amphetamines in 1985 with no sanctions, only treatment. Testing started again in 2006, this time with sanctions for the second, third, or fourth positive test (6). As a result, data that help monitor the issue started flowing in. Players can request therapeutic use exemptions (TUEs) allowing them to be treated with a prohibited substance if it is for valid medical reasons (7). In 2007, 2008, and 2009, the number of TUEs for treatment of attention deficit hyperactivity disorder (ADHD) with amphetamine or similar substances was 103, 106, and 108, respectively, with 108 representing about 9% of major league players (7). For comparison, the National Institute of Mental Health estimated that 4.4% of American adults ages 18–44 had ADHD symptoms in 2006 (7). Whether these data indicate that amphetamine use in MLB is still problematic or simply reflects difficulty in estimating the prevalence of ADHD is yet to be determined.

Screening for any target drug requires separating it from hundreds of other substances in the biological fluid sample, then identifying it. Urine analysis for amphetamine provides an excellent example of the use of hyphenated mass spectrometry for an undergraduate analytical chemistry course. Of the 30-some worldwide anti-doping laboratories, many are moving routine screening for stimulants, including amphetamine, from GC-MS to more sensitive LC-MS methods. Following sample preparation, the "front end" of the method usually employs reversephase HPLC. The extraction and chromatographic conditions exploit a wealth of chemical equilibria, including the use of SPE cartridges for sample preconcentration and the use of pH control and ion-pairing reagents to effect separation and reduce

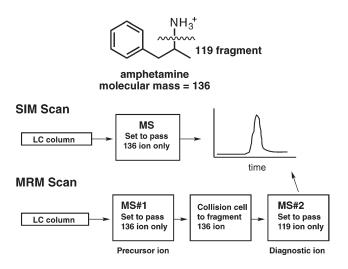


Figure 1. The structure, LC-MS, and LC-MS-MS of amphetamine.

peak tailing for polar analytes such as amphetamine (8, 9). These topics are covered in most modern analytical texts (10).

The mass spectrometer acquires scan after scan continuously. The goal of the targeted screen is merely to determine whether amphetamine might be present. Because the reference spectrum of amphetamine and characteristic fragment ions are known, there is no need to acquire complete mass spectra in the full-scan mode. Better acquisition strategies are illustrated in Figure 1.

In the selected ion monitoring (SIM) mode, one mass spectrometer can be set to pass only one characteristic ion of the analyte. Electrospray ionization adds a proton to amphetamine, forming a characteristic M+1 ion at m/z 136 (11). If there is an HPLC peak at the expected retention time for amphetamine, then amphetamine is detected. A SIM scan is more selective than a full scan because SIM allows chromatographic overlap to be resolved by the mass spectrometer, as long as the target analyte's characteristic ion is absent from the coeluting component(s). Moreover, because the mass spectrometer scans fewer ions in a SIM scan than in a full scan, the acquisition time per ion is longer for the SIM scan, resulting in better signal-to-noise ratios and lower detection limits.

Another scan mode, called multiple (or selected) reaction monitoring (MRM or SRM) mode, is possible with tandem mass spectrometry (MS-MS), using two mass spectrometers and a central collision cell (Figure 1). For amphetamine, the first MS can be set to pass only m/z 136 (the precursor ion) and the second MS can be set to pass only a diagnostic fragment ion (m/z 119) produced by impact of the precursor ion with an inert gas in the intermediate collision cell. MRM is far more selective than SIM because one ion must fragment and yield the other to produce a chromatographic peak.

Other important analytical concepts, such as the use of internal standards, establishment of recoveries, detection limits, linearity of signal, and method validation, can also be addressed using amphetamine analysis by LC-MS (8, 9).

Anabolic Steroids

Anabolic steroids include testosterone (Figure 2), a natural hormone, and its endogenous and synthetic analogues. Anabolic steroids can turn on cells to produce protein, leading to the

Figure 2. The structure of testosterone.

development and repair of muscle tissue (12). Athletes believe that steroids allow them to do more frequent heavy workouts, which, when combined with proper diet, can lead to improved strength and performance. Franke and Berendonk have examined files made public after the collapse of the German Democratic Republic (GDR) that show how the GDR state-supported program of steroid use, especially for women athletes, produced significant gains in athletic performance between 1966 and the mid 1980s (13). In addition, the Mitchell Report on PED use in MLB (6) and recent books have documented the use of steroids by contemporary athletes (14, 15). Steroid use can have adverse effects on numerous organs. beyond the reproductive system.

WADA prohibits all anabolic steroids and names about 70 examples. Laboratories screen for them by hyphenated mass spectrometry. An article by Trout and Kazlauskas provides an excellent comparison of the use of GC with low and high resolution mass spectral detection for the screening of steroids (16). This article also provides some interesting and relevant chemistry in the sample preparation step, including the use of a derivatizing reagent to enhance steroid volatility. Thevis et al. describe the use of LC with tandem mass spectrometry (LC–MS–MS) to screen for anabolic steroids (17).

One way to try to beat the test is to use a substance that laboratories do not screen for; this approach was uncovered in the greatest sports doping scandal in U.S. history: the BALCO case (14). In this example, the "designer" steroid (an untested, unapproved, potent drug) was not monitored in the normal steroid screen because its existence, and thus, its chromatographic and mass spectral parameters were unknown to the world. An athlete using this designer steroid was in "the clear" because its use would not trip a positive test; indeed, the BALCO code name for this substance was "The Clear" (14). How the identity of The Clear was discovered provides a fascinating classroom anecdote involving serendipity and subsequent chemical detective work using mass spectral and other data for its structure determination.

Things came apart for BALCO because Trevor Graham got angry. Graham, a high-profile sprinter coach, was convinced that certain track athletes were getting an unknown steroid from Victor Conte, the owner of BALCO. Graham obtained a used syringe, which he once claimed he pulled out of the trash at a track meet, and sent it anonymously to the U.S. Anti-Doping Agency (USADA) in Colorado Springs. USADA immediately sent a methanol rinse of the syringe to Don Catlin and his staff at the UCLA Olympic Analytical Laboratory for identification of the substance it had contained (14, 18).

A summary of the approach used by Catlin's team to determine the structure of The Clear from mass spectral data is shown in Figure 3. Commonality of fragment masses for The Clear and gestrinone, a known anabolic steroid, indicated that the two must have similar structures. Observation of ions

Figure 3. THG: The Clear (18) structure and common mass spectrum ions

differing by four mass units in the upper mass range suggested that The Clear had a molecular mass four units higher than that of gestrinone, which the investigators realized could result from hydrogenation of the acetylenic group on gestrinone. When a sample of gestrinone was hydrogenated by Catlin's team, the mass spectral and NMR data of the resulting product matched those of the substance from the syringe (18). Therefore, the substance was called tetrahydrogestrinone or THG. This result, as part of the evidence gathered in a larger federal investigation, eventually led to the conviction of several individuals, including Victor Conte and the creator of THG, Patrick Arnold, for the distribution of illegal PEDs to athletes (19). Graham's decision to slip the recovered syringe to USADA was listed as one of 10 history-altering decisions of the past decade in the November 30, 2009 issue of Newsweek magazine. Ironically, Graham was ultimately convicted for lying about his own contacts with a PED dealer to a federal grand jury investigating the BALCO case (20). The identities of two other designer steroids, norbolethone and madol, were also discovered by Catlin's team and provide additional examples of the application of hyphenated mass spectrometry for compound identification (21, 22).

Although the appeal of designer steroids to avoid detection is evident, athletes often turn to testosterone for performance enhancement, in part, because it, too, presents testers with a challenge: how does one differentiate between endogenous (natural) and exogenous (synthetic) testosterone in an athlete's urine? Simply measuring testosterone (T) will not work because individual levels vary widely. Instead, testers take advantage of a "quirk" of nature involving the human body's production of an epimer of testosterone called epitestosterone (E). The two epimers differ only in the configuration of the -OH group at the C17-position of the steroid structure (Figure 2). Whereas T functions as an anabolic steroid, E does not, thereby providing an excellent classroom example of the dependence of function on subtle structural differences. Most normal men have a ratio of the T to E concentrations in urine (T/E ratio) of about 1:1. Less common values range from below 0.1 to above 3. The T/E ratio can readily be determined by GC-MS (23).

Because the T/E ratio increases temporarily after T is taken, it is used to screen for T abuse, with a threshold of 4 for reporting findings (24). There are two problems with any threshold value. The first is that rare, drug-free individuals might have a naturally elevated T/E. The second problem is that T users might "fly under the radar" either by taking microdoses of T to keep their

T/E ratio just under 4 or by taking both T and E. BALCO sold such a mixture as "The Cream", to be rubbed on the skin (14). T users can be distinguished from nonusers by plotting T/E as a function of time over weeks or months: values are expected to be stable for nonusers, but in users, they shoot up after T is used, then drop back down (23).

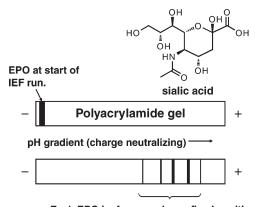
To find a better way to tighten T/E loopholes, testers focused on the carbon skeleton of T because there is a difference in ¹³C content between natural and synthetic T. Roughly 1.1% of all carbon is ¹³C, but synthetic T contains less ¹³C than does natural T because they arise from different biosynthetic pathways. From the moment atmospheric carbon dioxide (CO₂) is fixed in plants by photosynthesis, and at every subsequent biosynthetic step in plants and animals up the food chain, some ¹³C is left behind because of the isotopic effect. By the time T is made either by humans, from biosynthetic or dietary cholesterol, or by pharmaceutical companies, from plant sterols, ¹³C has been depleted to different extents along the two different pathways.

The technique used to detect the difference is gas chromatography—combustion—isotope ratio mass spectrometry (GC—C—IRMS), also known as carbon isotope ratio (CIR) testing. The anabolic steroids present in urine are extracted and separated by GC. When T exits the GC, it goes through the combustion oven (-C-), where pyrolysis converts every carbon atom to either $^{12}\mathrm{CO}_2$ or $^{13}\mathrm{CO}_2$. The IRMS measures the abundance of isotopic variants of CO₂ and calculates the $\delta^{13}\mathrm{C}$ (delta) value, which is the difference between the $^{13}\mathrm{C}/^{12}\mathrm{C}$ ratio of the sample and that of an international standard. The unit is ‰ (per mil). By definition, the delta value of the international standard is 0 ‰. Examples of values might be -23‰ for natural T and -30‰ for synthetic T. The values are negative because both compounds contain less $^{13}\mathrm{C}$ than the international standard: 30 fewer parts per thousand for the synthetic T.

A CIR test on a single urine sample with T/E > 4 can lead to adjudication decisions with no need to collect more samples. The greatest power of CIR testing, though, is that its most sophisticated version can also detect the abuse of T precursors and metabolites, even if the exact compound taken is never identified. For example, in 2009, U.S. cyclist Tyler Hamilton tested positive for "testosterone or its precursors." He explained publicly that he had taken a supplement containing dehydroepiandrosterone (DHEA), which is prohibited in sports as an anabolic steroid because it is a precursor of T in the human body (25). CIR testing makes it possible to detect steroid use even in the absence of an elevated T. For instance, at the 2006 Tour de France, CIR tests on Floyd Landis' urine samples from four stages detected evidence of doping even though their T/E ratios were not greater than 4 (26). Finally, WADA rules allow anti-doping programs to sanction athletes based solely on CIR results. Therefore, CIR is truly a novel approach in the detection of doping in sports (27, 28).

Erythropoietin

Erythropoietin (EPO) is a protein hormone that is released from the kidneys and acts on the bone marrow to stimulate red blood cell (erythrocyte) production. This increases hemoglobin levels and the quantity of oxygen carried to muscles, thereby enhancing stamina and endurance. On the downside, EPO also increases blood viscosity, which can lead to greater risk for heart attacks and strokes. Human EPO (uEPO) is a 30.4 kDa



Each EPO isoform reaches a fixed position when gradient pH equals pI for the isoform.

Figure 4. IEF of EPO.

glycoprotein (protein—sugar conjugate) containing 165 amino acids and 4 carbohydrate chains. Each of the carbohydrate chains can vary in length, branching pattern, size, and composition. These chains contain variable numbers of sialic acid groups. Thus, EPO consists of a collection of "isoforms" that have the same protein but vary in total negative charge due to different numbers of sialic acid residues (29).

The potential for pharmaceutical EPO to eliminate the need for transfusions for conditions such as anemia, where oxygen levels are reduced, led to the creation of recombinant human EPO (rEPO) in the mid-1980s. Amgen began manufacturing Epoetin α in the late 1980s, and it became the first blockbuster drug produced by biotechnology. Athletes, especially in endurance sports such as cycling and cross-country skiing, were well aware that performance could be enhanced by increasing blood oxygen levels. By the late 1980s, the use of EPO as a PED began to replace more complicated and potentially dangerous transfusions as a form of "blood doping" (30). Clinical studies have shown that EPO can produce a 5-10% gain in aerobic performance (30). An example of the impact of rEPO on sport can be inferred from the mean hemoglobin levels in elite male cross-country skiers, which went from below to above that of the reference population between 1989 and 1997 (31).

Testing for rEPO presents a dilemma similar to that for testosterone: how does one differentiate between endogenous EPO (uEPO) and exogenous EPO (rEPO)? EPO can be characterized by its isoform distribution using a technique called isoelectric focusing (IEF) (32). This technique is illustrated in Figure 4. An EPO sample is applied to one end of a polyacrylamide gel containing a pH gradient. An electric field is applied across the gel, and the negatively charged EPO isoforms migrate toward the positive end. As they move along the pH gradient, charge is neutralized until each isoform reaches the point where the gel pH equals the isoelectric pH (pI) of the isoform. At its pI, by definition each isoform has an average charge of zero. Thus, it can no longer be affected by the electric field, and it stops moving. The result is a pattern of bands whose number, location, and relative intensities correspond to the various isoforms that make up EPO (Figure 4). This pattern is ultimately visualized by chemiluminescence (33).

IEF can be used to distinguish uEPO from rEPO because the two are not identical. uEPO is produced in human kidney cells, whereas the original rEPO (Epoetin α) is produced in

Chinese hamster ovary cells (30). The extent of sialic acid addition is cell-dependent. Consequently, the IEF patterns are different, as seen in Figure 1 of the WADA Technical Document TD2009EPO (34). When "follow-on" manufacturers produce rEPO, they might use different cell lines or production, purification, and formulation processes (30). As a result, "follow-on" rEPOs are not identical to Epoetin α ; therefore, they are not called "generics" but "biosimilars." In addition, improved, longer-lasting versions of EPO (NESP, CERA) are now available. Thus, analytical methods need to discriminate between uEPO and a growing number of recombinant products.

The "cat-and-mouse game" between dopers and testers is nicely illustrated by examples of IEF testing for EPO. Athletes have a knack for getting their hands on new versions of PEDs even before these drugs are approved for medical use, in part because these athletes hope that a reliable drug test will lag behind the PED's introduction. Such was the case when Amgen introduced a "second generation" EPO called darbepoetin α (NESP, Aranesp) shortly before the 2002 Winter Olympics. As is seen in Figure 1 in the WADA Technical Document TD2009EPO, the IEF patterns of uEPO and NESP differ substantially (35). This is what allowed Catlin's team from UCLA to identify NESP in the urine of three athletes at those games, and all three received sanctions (35). More recently, a "third generation" EPO (CERA or Mircera) was found in the stored blood samples of athletes from the 2008 Tour de France and 2008 Olympics in Beijing (36). Figure 1 in the WADA Technical Document shows why this is possible using IEF testing (34). The test, which was developed by laboratories with support from Roche, was not ready for use at competition time, but sample storage is a powerful way to extend the reach of testers. CERA is a rare example of a PED that is easier to detect in blood than urine because very little of it is excreted.

Although Epoetin α, NESP, and CERA can readily be distinguished from uEPO by IEF, this may not be the case for some EPO biosimilars. An estimated 80 rEPO biosimilars may be sold in emergent countries (37). The WADA criteria for declaring a positive rEPO finding from IEF patterns may not be met by all of these EPO variations. To close this potential loophole, WADA recently approved, for supplemental identification, a technique often discussed in the undergraduate curriculum: sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (38). SDS-PAGE and IEF share the use of an electric field to drive analytes through a polyacrylamide gel. However, with SDS-PAGE, separation is not by charge but by size (mass). A protein mixture is exposed to sodium dodecyl sulfate (SDS), therefore denatured, and then coated with dodecyl sulfate anions. This produces a total negative charge approximately proportional to protein size (39). The mixture is then separated by electrophoresis through a sieving gel that retards larger molecules more than smaller ones. Figure 2 in the WADA Technical Document TD2009EPO shows that SDS-PAGE can be used to distinguish uEPO from biosimilars (34). Of course, this method works only when there is a sufficient mass difference between uEPO and a given biosimilar.

Human Growth Hormone

Human Growth Hormone (hGH) is a 191 amino acid protein hormone produced in the pituitary gland. hGH induces the liver to secrete insulin-like growth factor (IGF-1), which in

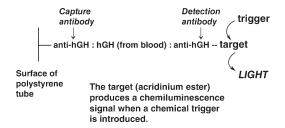


Figure 5. WADA-approved test for hGH.

turn stimulates bone, muscle, and organ growth (40). Thus, hGH essentially makes "everything grow". It also regulates the body's ratio of lean muscle mass to fat. Recombinant rhGH has legitimate medical uses, including the treatment of growth disorders and muscle-wasting disease accompanying AIDS. rhGH is the most enigmatic of PEDs. Araton in *The New York Times* has called it "the elephant in the enhancement arena" because of its rumored widespread use and the fact that detection of rhGH is highly problematic (41).

Until recently, hGH enhancement of athletic performance was not supported by the scientific literature, but this conclusion is now challenged by the work of Meinhardt et al. (42). These authors administered hGH alone and hGH combined with testosterone in a randomized, placebo-controlled study with nearly 100 recreationally trained athletes. Their results showed a 3.9% increase in sprint capacity from hGH, which, for men, increased to 8.3% when hGH was combined with testosterone. The increase with hGH alone translates into about a 0.4 s improvement over 10 s in a 100 m dash, which is greater than the difference between the first and last place finishers in the 2008 Beijing Olympics. Moreover, combining rhGH with low levels of testosterone may provide athletes with a significant "boost", while keeping the T/E ratio below the threshold of 4 (43). Although measures of strength and power did not show improvement with hGH in Meinhardt's study, the authors suggest that the dosages they used may be lower than those used by competitive athletes.

The levels of the hormone in urine are quite low, less than 1% of its levels in blood. Therefore, detection of rhGH in urine is not currently possible, and the only WADA-approved method for rhGH requires a blood sample. Similar to EPO, natural hGH consists of a mixture of isoforms. These differ in length and, therefore, mass. Whereas hGH consists mainly of three isoforms with masses of 17, 20, and 22 kDa, rhGH contains only the 22 kDa isoform, identical to its natural counterpart. To detect the difference in isoform proportions resulting from rhGH use, the test method employs two assays in parallel: one to quantitate the 22 kDa isoform and a second to quantitate all other isoforms. Athletes who use rhGH are expected to show an usually high "isoform ratio" of the 22 kDa isoform to all other hGH isoforms (44).

The test method is based on the use of antibodies: proteins produced by an animal's immune system in response to a foreign substance (an antigen). When hGH is the antigen, an animal produces anti-hGH antibodies, which are powerful analytical reagents because they bind strongly and selectively with the antigen (hGH). This is the basis for a technique called immunoassay, which is covered in analytical texts (45). The principle of the immunoassay for hGH is illustrated in Figure 5. Anti-hGH antibodies that bind preferentially with the 22 kDa isoform ("capture antibodies") are isolated and attached to the inside

surface of a polystyrene tube. When blood serum containing hGH is introduced, the 22 kDa hGH is captured preferentially by binding with the antibody. The tube is washed to remove unbound molecules, and a second anti-hGH antibody ("detection antibody") is added to form a sandwich complex with the bound hGH. The tail of the second antibody consists of a compound that can be triggered chemically to emit chemiluminescence for detection. The tube is washed to remove unbound molecules. The chemical trigger is added. Chemiluminescence occurs only if hGH is present in the sample to form the sandwich complex, and its intensity can be used for 22 kDa hGH quantitation. To quantitate all other isoforms in the same sample, the same approach is used with capture antibodies that bind preferentially all other isoforms (44).

This method was used at the Olympics in 2004 (Athens), 2006 (Turin) (45), and improved in 2008 (Beijing), but no positive test was reported (46, 47). The first ever (48) and second positive tests for hGH were reported in 2010. rhGH detection is difficult in part because it is excreted from the body in only 24-36 h. Given this limitation of the WADA-approved test and the ubiquity of rhGH use, there is considerable interest in improving its detection. Alternative, indirect approaches look at "downstream" effects of rhGH administration by measuring specific biomarkers that are released when rhGH is used. One approach uses immunoassays to measure a hormone (insulin-like growth factor-1, IGF-1) and a peptide (procollagen III peptide) whose serum concentrations are increased by rhGH (49). A second approach uses LC-MS-MS to identify IGF-1 and another marker of rhGH use, leucine-rich α-2-glycoprotein (LRG), by detecting their fragments after enzymatic hydrolysis (50). Both methods have good sensitivity and the period of detection should be longer than that of the isoform ratio method.

The ultimate for rhGH testing would be a urine test, less invasive than the current blood test. As of October 2010, the only major American professional sport league that tests players' blood samples for rhGH is MLB in its Minor League Program; the NFL and MLB (for its Major League Program) are considering it. Developing a urine test is hugely problematic, given the low levels and short time rhGH would appear in urine. Fredolini et al. have taken an interesting approach using nanotechnology. They have shown that hydrogel nanoparticles functionalized with Cibacron Blue F3G-A dye can trap all of the hGH in a urine sample (~30 mL) (51). The hGH can then be released into a much smaller sample volume, at a much larger concentration suitable for immunoassay. Although this is encouraging, most experts believe that a urine test that can differentiate hGH and rhGH is still years away.

Longitudinal Monitoring

In the same manner as physicians, who monitor individual patients' weight, cholesterol, and chest X-rays to spot changes from an individual's baseline profile as potential signs of disease, testers monitor biomarkers, such as urinary endogenous steroids and blood readings, watching for telltale deviations due to steroid abuse or blood doping. This is called longitudinal testing. It is an example of indirect detection because it does not directly identify the drug taken. Many kinds of biomarker data together can undergo trend analysis, using complex mathematical models that help detect deviations; however, it is not possible to estimate the probability that a deviation is due to doping. A deviation is merely an unusual occurrence that might lead to further

Table 1. Summary of PED Detection Methods

Performance-Enhancing Drug	Analytical Detection Methods
Stimulants	LC-MS LC-MS-MS
Anabolic Steroids	GC-MS (low and high resolution) LC-MS-MS GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) = Carbon Isotope Ratio (CIR)
Erythropoietin (EPO)	Isoelectric focusing (IEF) SDS-PAGE
Human Growth Hormone (hGH)	Immunoassays Biomarkers LC-MS-MS

investigation, such as retesting existing samples or targeting the athlete for future tests (52). Therefore, longitudinal testing will never replace conventional doping control tests; it augments them. The WADA's Athlete Biological Passport Operating Guidelines (53) and Union Cycliste Internationale (UCI) Biological Passport (54) include longitudinal testing and trend analysis. As of October 2010, the UCI has found recombinant EPO in the urine samples of six riders target-tested on the basis of passport blood data using blood biomarkers such as hemoglobin level and percent red blood cells (55). This shows that longitudinal monitoring helps to detect dopers.

Conclusion

The topics covered are summarized in Table 1 and represent only the major contemporary PEDs and detection approaches. The WADA prohibited list includes many more categories, such as masking agents and methods. The next challenge on the horizon for testers is gene doping. This is an offshoot of gene therapy research, whereby an individual's genes are modified, deleted, or substituted to treat disease. Technically, this is not difficult, but clinical trial outcomes have been highly problematic and even fatal (56). Nonetheless, the appeal to athletes of a gene modification that could, as examples, enhance uEPO or IGF-1 levels is very strong (57). Could such manipulations be detected? WADA has hosted several symposia to address this issue in a proactive manner but has not yet approved any method to detect gene doping (58).

Chemists face formidable challenges in the "cat-and-mouse game" with those who enable PED use among athletes. This provides opportunities to bring daily headlines into the chemistry classroom, thereby placing important chemical concepts and methods in a social context. Jacques Rogge, president of the International Olympic Committee, has pointed out that "Doping is to sport what criminality is to society, and there will always be criminality in society" (59). In this regard, the topic has parallels with forensic chemistry as a source of interesting and relevant pedagogical examples to enrich and deepen the classroom experience. And if sports examples enhance interest in chemistry, the reverse is also true. One young woman, at the end of the "Chemistry and Athletic Performance" course, stated that she now regularly reads the sports pages because "that's where the scandal is". Hopefully this kind of overlap will inspire young researchers to help fight drug abuse in sports and society.

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