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The Abuse of Chemistry in Doping

When we think of sports, the words resilience, passion, training, and dedication come to mind. But it is unfortunate that many great results and new records have recently been tainted by the risk of doping. In sports, doping refers to the use of performance-enhancing drugs, a term that has come to plague athletes from all competitive arenas. Organizations regulating sporting competitions are increasingly faced with athletes that have succumbed to the allure of fast results provided by various doping methods. Though doping refers predominantly to the use of drugs and can be detected through various physiological tests, it is the threat of blood doping and gene doping that, besides the difficulty in detection, poses new ethical and moral questions to all those involved in the highly competitive atmosphere of modern sport. The chemical sciences play a very important role in anti-doping research and education. Chemistry is fundamental component of analytical techniques that seek to eradicate the unlawful use of drugs and other doping methods. Gaseous phase chromatography, high pressure liquid chromatography, and mass spectrometry are only few of the tests used worldwide in 33 laboratories accredited to perform doping analyses of athletes.

History of Doping in Sport

The use of various substances to enhance one's sporting performance is as old as competitive sport itself. West Africans used *Cola acuminita* for running competitions. The ancient Greeks used special potions of plants and ate hallucinogenic fungi to become fitter. In the Roman era, stimulants were used by gladiators to make them fight in a more

spectacular way and to overcome fatigue. Professional sport and spectator sport re-emerged only at the end of the nineteenth century, when *dop* first appeared in the English dictionary, referring to a narcotic mixture of opium used for racehorses. Doping re-emerged as well, and modern chemistry catalyzed doping practices that would last us to the 21st century. It started with alcohol, caffeine, and progressed to strychnine and cocaine- substances often used in the 19th century to alleviate fatigue. In effect, the athletes became test subjects for the physiology of stress and the substances that would improve alertness. Strychnine was most dangerous as it is a highly toxic poison (LD_{50}=10mg), but that did not prevent athletes and their entourages from administering doses of strychnine as a stimulant.[1] Strychnine acts as an antagonist of a ligand-gated channel in the spinal cord and brain, and is used widely as a pesticide for small vertebrates. The best known case regarding the use of strychnine is the American Thomas J. Hicks, who won the St. Louis Olympic marathon in 1904. Hicks was administered strychnine by his trainer, which proved to be advantageous to athletes competing in long races. The dosage for medical use was between 1.1 and 6.4 mg; the maximum dosage used was 3.2 mg. Hicks' doctor, Dr. Charles Lucas, commented "that the Marathon race, from a medical standpoint, demonstrated that drugs are of much benefit to athletes." Before this time, the ethics of using drugs to enhance physical performance had not yet entered the public conscience.

Tour de France

It was the 1998 Tour de France that stirred the most controversy over doping scandals, and eventually led to the development of the World Anti-Doping Agency, or WADA. Large amounts of doping products were confiscated from the vehicle of the

French Festina cycling team before the start of the race. The discovery of anabolic steroids, erythropoietin, and syringes led into further doping investigations and ultimately, ten people associated with the team stood trial. When the circumstances repeated themselves in the 2000 Tour de France after three cyclists tested positive for erythropoietin, a hormone that stimulates red blood cell production, Judge Daniel Delegove, who presided over the trial, commented, "These are not racers, they are pedaling test tubes." When recombinant erythropoietin, r-EPO, became popular in cycling, the average speeds of winners of the Tour de France significantly increased. As Graph 1 shows, from 1991 to 2004, average speeds jumped by nearly 8%, an increase that cannot be explained solely by the use of more advanced equipment and training.[2]

The BALCO scandal

The competitive nature of sports has fueled the use of steroids by professional athletes, which have been linked to such elite athletes as Barry Bonds, a major league baseball player, and Marion Jones, an Olympic gold medalist in track and field. The reputation of these athletes has been tainted due to their use of the steroid Tetrahydrogestrinone, also known as THG or "the clear," an anabolic steroid banned by the Food and Drug Administration in 2003.[3] THG is a highly potent agonist for the androgen and progesterone receptors and acts by stimulating growth of muscle tissue. THG is administered intramuscularly, and increases skeletal muscle production by boosting the transcription of RNA to protein. Prolonged use can cause side effects such as infertility in men and women, acne, excessive hair growth, and immunosuppression. Although the use of androgenic steroids is banned in competitive sports, chemical modifications of steroidal structure have allowed the development of new steroids not

named on the banned list. THG was a novel steroid when it first appeared; it was never marketed so information regarding its hormonal properties was not known. Developed by chemist Patrick Arnold of the Bay Area Laboratory Co-operative (BALCO), a nutritional supplement company, THG, the then un-detected drug, was made available to several high-profile athletes. The drug was chemically manufactured from hydrogenation of gestrinone via catalyzation by palladium-charcoal. Gestrinone is a synthetic steroid hormone used for treatment of endometriosis, a condition in which the endometrium, the tissue lining the uterus, grows outside of the uterus, spreading into the pelvis. Tetrahydrogestrinone was made public in 2003, when U.S. sprint coach Trevor Graham made an anonymous call to the United States Anti-Doping Agency (USADA) accusing athletes for doping using the undetectable steroid. As evidence, Graham provided the agency with a syringe containing THG which was used to develop appropriate testing methods. Barry Bonds and Marion Jones both claimed that they had taken THG unwillingly at the advice of their coaches. Bonds has been indicted for perjury and obstruction of justice, while Jones has admitted to using THG and was stripped of her Olympic medals.

The fight against doping: WADA

The World Anti-Doping Agency, or WADA, is an independent organization founded by The International Olympic Committee (IOC) in 1999 designed to combat the use of drugs in sports. The organization is responsible for implementing testing procedures for international sporting federations and provides a list of forbidden substances for athletes. WADA was funded in entirety by the Olympic Movement during its first two years, totaling $18.3 million dollars. In January 2002, co-funding was

implemented with Governments providing 50 percent and the Olympic Movement 50 percent of funds for WADA projects. The WADA 2008 World Anti-Doping Code provides a list of prohibited substances and doping methods from competition, illustrated in Table 1.[4]

There is often confusion about the need to address athlete illnesses while preserving their competitive integrity. Should an athlete require medication that is under the Prohibited List to treat a particular illness, a Therapeutic Use Exemption authorized by WADA can allow an athlete to take the needed medicine. The criteria for the exemption are that 1) the athlete would experience significant health problems without taking the prohibited substance, 2) the therapeutic use would not produce significant enhancement of performance, and 3) there is no reasonable therapeutic alternative.

Blood doping

Blood doping is a method used by many athletes in endurance sports to increase their VO2 max, or maximal aerobic power, by increasing the number of red blood cells. There are approximately 280 million hemoglobin molecules in a red blood cell, essential for oxygen transport from lungs to muscles. Hemoglobin levels can rise through infusion of erythrocytes or whole blood, increasing the oxygen-carrying capacity of the blood, thereby improving physical performance. Unfortunately, there is no practical method available to combat blood doping in sports. There are sophisticated DNA techniques that monitor the increase in hemoglobin, increased iron, and reduced plasma erythropoietin concentration following infusion, but the procedures are only effective if samples are available from before and after the infusion. A hormone that is increasingly manipulated by athletes is erythropoietin (EPO), a glycoprotein that enhances the production of red

blood cells in relation to decreased oxygen availability. Clinical studies have shown that EPO can provide a 5 to 15% boost in athletic performance via production of extra red blood cells to improve oxygen uptake and thus aerobic power. However, lethal side effects of recombinant erythropoietin use (r-EPO) include blood clots, heart attack and stroke. Today, genetically engineered EPO is available via recombinant DNA techniques, a potent drug that has helped individuals suffering from diseases such as anemia. Researchers at Amgen developed r-EPO and began selling it in 1989 for kidney dialysis. Amgen's Epogen drug had sales of $2.6 billion in 2004. However, EPO is also the prime candidate for doping. The appeal lies in the fact that EPO is identical to natural hormones in the body, making it difficult to detect by standard analysis methods. Enzyme immunoassays can measure serum EPO levels, but the tests are unable to determine if the EPO is produced naturally or injected by athletes. The World Anti-Doping Agency has now implemented combination urine and blood tests to detect EPO abuse by athletes.

Gene doping

Gene doping can be considered a very clever chemistry. Since its advent in the late 1960s and early 1970s, gene therapy has been a controversial science, and has become a topic of growing ethical concern in the athletic community. There is a fear that gene therapies used for the treatment of human genetic diseases can be advantageously used by athletes to augment normal human functions in order to enhance performance. Gene doping uses gene transfer technology to enhance physical traits such as muscle size and strength, blood circulation, and efficiency of energy utilization. Two principal methods have been utilized to introduce gene transfer vectors into patients: the *ex vivo* and the *in vivo* methods. The *in vivo* method involves introducing the gene transfer vector

directly into the tissues of the patient. Injection of DNA into skeletal muscle is an effective way to cause genetic modification of muscle cells and offer continuous expression of the foreign gene. For example, injection of the gene that codes for insulin-like growth factor-1 (IGF-1) causes skeletal muscle to become hypertrophic and to contract with greater force and repair from injury more quickly. The *ex vivo* method involves addition of the gene transfer vector into the cells of the patient in the laboratory; these genetically corrected cells are then reintroduced into the patient. Another *ex vivo* approach involves culturing skin cells obtained from patient, genetically modifying them and then reintroducing them back into various tissues. Gene doping is a dangerous but attractive method for athletes because of the difficulty to be detected, but its application blurs the line between therapy and enhancement. By researching the physiological responses to gene transfer technology, at cellular and systemic levels, scientists are developing detection methods for gene doping based on knowledge of gene technology, immunology, transcriptomics, proteomics, and biochemistry. These chemical tools are vital for agencies regulating competitions to monitor athletes and establish proper tests to detect the misuse of genetics.

Testing for doping in sports

An effective drug-testing program is necessary to combat the abuse of drugs in the sporting arena, thereby creating a new forensic science. Clinically, blood level of a drug has been used for most tests of doping, as it more directly correlates with drug action than urinary level. But when utilized, a urine test can be positive due to unpredictable variables such as urinary output and fluid intake. However, the only permitted testing method for doping is urine analysis; a urine sample requires a non-

invasive procedure and drug/metabolite concentrations are frequently higher in urine than in blood, rendering a longer window time for detection.[5] Various chemical screening methods are also used to test for drug use in sporting competitions. These methods include gas chromatography, high performance liquid chromatography, immunoassays, and enzyme-linked immunosorbent assay methods. Gas chromatography uses a urine sample to transform the drug/metabolites of the sample into a gaseous state without destructing the molecule. The retention time, or the time that the substance takes to travel through a column, is a key component to revealing the identity of the substance. A mass spectrometer attached to the end of the column provides an ion fragment fingerprint of the substance. By matching the data to that of the authentic substance or to a sample from someone who has taken the drug, one can accurately identify a positive result. Liquid chromatography operates on the same principles of analysis as gas chromatography except that the sample is passed through the column in a liquid state. This is the preferred form of analysis because the sample can be analyzed under mild conditions and liquid chromatography can detect the presence of large molecular weight hormones such as EPO.

The future directions in drug testing also include hair analyses in addition to urine assays. Table 2 compares the drug detection methods using hair or urine analysis.[6] The hair of a drug user can indicate chronic use of drugs when an older section of the hair is analyzed. Clinical analyses have detected testosterone, cocaine, and other metabolites in human hair as they are excreted. Chromatography procedures for analysis are most power due to their great separation ability and detection sensitivity. In contrast with urine, hair analysis can be used for a long detection period and elucidates the pattern of potential

drug use. The International Olympic Committee does not employ hair analysis; however, the technology is becoming accepted in most courts of Justice.

Conclusion

As the chemical sciences uncover the mysteries of health and disease, the tools they provide can be used by athletes to improve their performance, potentially creating a world of tainted athletes. Thus, anti-doping research and education is fundamental to eradicating the use of drugs and doping methods in professional sports. The United States Anti-Doping Agency (USADA) budgets about $2 million per year to support research meant to deter athletes from doping. The chemical sciences must fulfill their role in developing effective drug testing methods to promote fairness in competition and ensure athletes are judged by their training and perseverance, rather than the strength of the drugs in their body.

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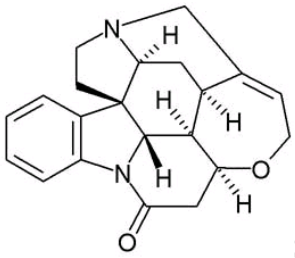
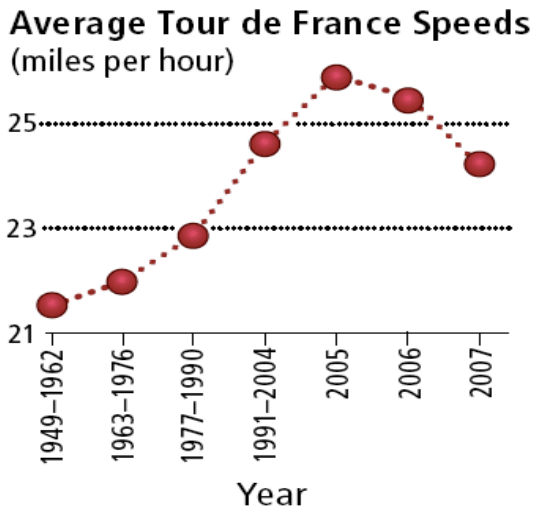
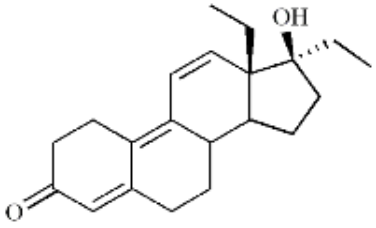
[1] 

Strychnine

[2] Graph 1: Average Tour de France Speeds in miles per hour



[3] 

Tetrahydrogestrinone, THG

[4] Table 1: List of prohibited classes and methods. For full list, visit:
http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf

Prohibited classes of substances
Stimulants
Narcotics
Anabolic agents
Anabolic androgenic steroids
Other anabolic agents (beta-2-agonists)
Diuretics
Peptide hormones, mimetics and analogues
Agents with anti-oestrogenic activity
Masking agents
Prohibited methods
Enhancement of oxygen transfer
Pharmacological, chemical and physical manipulation
Gene doping
Classes of prohibited substances in certain sports
Alcohol
Cannabinoids
Local anaesthetics
Glucocorticoids
Beta-blockers

[6] Table 2 compares testing for doping in sport via hair or urine analysis

Parameters	Urine	Hair
Drugs	All, except some peptide hormones	All, except hormones
Major compound	Metabolites	Parent drug
Detection period	2–5 days, except anabolic steroids	Weeks, months
Type of measure	Incremental	Cumulative
Screening	Yes	No
Invasiveness	High	Low
Storage	−20°C	Ambient temperature
Risk of false negative	High	Low
Risk of false positive	Low	Undetermined
Risk of adulteration	High	Low
Control material	Yes	Needed