Doping in Sport: Misuse, Analytical Tests, and Legal Aspects

As part of the International Congress of Clinical Chemistry, a symposium, Doping in Sport: Misuse, Analytical Tests, and Legal Aspects, was held, a week before the start of the Olympic Games at Atlanta, bringing together a number of international experts concerned with various aspects of controlling drug abuse in sport. The presentations focused particularly on anabolic-androgenic steroids (AAS), these analytes still comprising the greatest number of positive findings in samples tested by International Olympic Committee (IOC)-accredited sports drug-testing laboratories. Although various aspects of AAS use and detection have been reported on in the last decade, the proceedings of this symposium, published in this issue of Clinical Chemistry, bring together health, social, endocrine, and analytical perspectives in a widely read peer-reviewed journal.

Those who think that the harmful effects of AAS administration are relatively benign and perhaps should not be banned by the IOC may find particularly sobering the article by Franke and Berendonk [1], which describes the health and social consequences of the clandestine state-sponsored program of administration of AAS in the former East Germany. This article should be extremely useful for educational purposes and, we hope, will encourage some present users to abandon the practice. The mechanism of action of AAS is still not well understood, and the article by Wu [2] is a useful update. Catlin et al. [3] discuss the pertinent issues of detecting AAS administration, in particular of testosterone (T), which is commonly abused. Stenman et al. [4] give a clinical chemistry perspective on the problems of detecting human chorionic gonadotropin (hCG) by immunoassay, the protein hormone that may be used by competitors to stimulate testicular production of T. Bowers [5] discusses analytical advances to detect performance-enhancing compounds, particularly with reference to xenobiotic AAS and peptide hormones, such as hCG.

But why bother about anabolic steroids? Attention began to focus on the steroid problem in modern-day sports with the allegation that Soviet weightlifters were administering T to gain strength in the early 1950s [6]. The problem became greater with the advent of synthetic analogs designed to enhance the anabolic properties. Nandrolone, the 19-nor analog of T, was the first compound to show enough myotrophic-androgenic dissociation in animal experiments [7] to justify its introduction in clinical therapy as an anabolic steroid [8]. With the development of nandrolone, studies on numerous substituted and hydrogenated analogs of T soon followed. Many US patents were awarded to drug companies around 1960, e.g., methandienolone to Ciba (1959), oxymesterone to Farmitalia (1960), stanozolol to Sterling Drug (1962) [9]. Clinically, there was great hope for the use of these drugs in promoting protein anabolism without evoking a strong androgenic effect. However, some sporting competitors also desired these drugs, in preference to T, for the same reasons. In 1967 the IOC Medical Commission banned the practice of doping in sport; at that time, anabolic steroids were not included in the banned class of compounds because there was no test for them. Nonetheless, use of anabolic steroids at international level was perceived to be rife; e.g., a decathlon athlete, Dr. Tom Wadell, told the New York Times that he estimated about a third of the US track and field team had used steroids at the pre-Olympic training camp before the Mexico City Games in 1968 [6].

With the successful introduction of an RIA screen for anabolic steroids, [10] and a GC-MS method for confirmatory purposes [11], a trial test was introduced at the Commonwealth Games in New Zealand in February 1974, targeting the orally active alkylated AAS. Of 55 samples, 9 failed the screen and 7 samples were confirmed to be positive. In April 1974, the IOC Medical Commission included anabolic steroids as a banned class of compounds. Since then, the number of samples tested has grown to >90 000 annually, the tests have evolved, and so has the sophistication of doping.

Ironically, the projected clinical usefulness of these hormones in reversing the catabolic state of patients, such as those with severe burns or wasting diseases, has not been realized. Consequently, many anabolic steroids have been withdrawn as licensed products in numerous countries, but a surplus of these steroids remains on the world market. AAS continue to be used as doping agents, despite a previous abundance of scientific literature supporting a lack of effect in intact men. For example, the extensive review of the literature by Ryan in 1976 [12] found a substantial body of evidence that these drugs do not contribute to muscle size and strength in healthy young men. More recent papers suggested that gains are possible if certain criteria are satisfied [13–15]. The new study by Bhasin et al. [16], using the advanced technique of magnetic resonance imaging [17], shows that administration of supraphysiological doses of T causes a significant increase in muscle mass in comparison with controls and that the effect of exercise is additive. This observation, combined with a significant increase in muscle strength, is powerful evidence and demonstrates that the athletes’ beliefs that AAS are effective were well founded.

The anabolic/anti-catabolic actions of androgens are discussed by Wu [2], who also points out that the orally active 17-alkylated steroids are potentially the most toxic. In these proceedings, Franke and Berendonk [1] describe several cases of liver dysfunction and severe damage from 17-alkylated steroids. In comparison, T is a much less harmful alternative, despite its androgenic properties. Bhasin and Bremner [18], in their review on the emerging issues in androgen replacement therapy, note that andro-
gen sales in the US appear to be growing 20–30% each year and that T is underutilized or inappropriately used for legitimate reasons while its use for unapproved indications continues to expand. Compared with finding xenobiotic steroids, the detection of doping with endogenous steroids still represents one of the greatest challenges to the drug-control laboratories. Although the 1980s heralded the introduction of an IOC-approved test for T administration [19], a large proportion of the test-positive samples continues to show an increased T to epitestosterone (E) ratio, the chosen marker of T administration.

Analytically, T administration is much more difficult to detect than doping with xenobiotic steroids, as Catlin et al. discuss in detail [3]. Franke and Berendonk [1] describe the “precompetition bridging programs” implemented in the former East Germany, where athletes would be switched from courses of xenobiotic steroids to T in the last few weeks before competition. Such a strategy continues to this day, but out-of-competition testing helps to preempt this changeover; such testing also helps to detect those who have already switched from xenobiotic steroids to large doses of T but plan to reduce their dose or stop administration a week or so before being tested at a competition. Even so, such is the sophistication of misuse that competitors are known to titrate their dose to below the T/E discrimination limit of 6, and others coadminister E with T in an attempt to “normalize” their T/E ratio. Indeed, as far back as 1982, when the T/E test was introduced, VEB Jenapharm began producing preparations of T and E propionate. There being no apparent pharmaceutical use for E, we are left with the conclusion that this former East German state-owned company was producing the formulation exclusively to beat the doping-control system. The determination of the $^{13}$C/$^{12}$C isotopic ratio of T [20–22] or more promisingly, of its 5a- and 5b-reduced metabolites [23], and comparison of this ratio with that of a metabolic precursor of T appears to offer a more specific confirmatory test than the T/E ratio.

Manipulation to beat the tests does not stop with the coadministration of E with T. HCG is used by some male competitors to stimulate testicular steroidogenesis. Because the testes are a major source of E [24], as well as T, the urinary T/E ratio remains unperturbed despite an increased concentration of plasma T [25]. More insidiously, if hCG is coadministered with T, the increase in the urinary T/E ratio is attenuated [26]. For these reasons, hCG was also designated as a banned substance by the IOC Medical Commission in November 1987. The IOC Medical Code requires that a validated immunoassay be used to detect and quantify hCG; for confirmation, a second, different immunoassay is required. This need to have properly validated immunoassays is stressed by Stenman et al. [4], because many assays also measure the hCG β-subunit (hCGβ) and have been validated only for serum rather than urine samples. Some assays cross-react with hCG core fragment, especially those designed to measure both hCG and hCGβ. Differing immunoglobulin specificity for gonadotropins is a recognized problem within clinical chemistry. An agreement on the broad specificity characteristics of antibodies to be used by the diagnostic industry could be a significant step towards improving standardization of measurement between laboratories [27]. Stenman et al. [4] suggest a number of criteria for doping control, which if met, leads these authors to conclude “that it should be possible to detect self-administration of hCG in men as reliably as anabolic steroids and T are now detected by mass-spectrometric methods.”

For doping control, can immunoprocedures be as reliable as mass-spectrometric methods? This is a crucial question, given the general acceptance that mass spectrometry is essential for evidential purposes. In the sports drug-testing laboratories, full-scan mass spectrometry is preferred for identification of small molecules and is likely to be rather more discriminating than is possible by using two or more immunoprocedures. However, far less consideration has been given to large molecules such as hCG, where highly specific immunoassays have been developed, as described by Stenman et al. [4]. Perhaps the most important question is, What is sufficient for the purpose? Currently, only immunoprocedures are adequate because mass spectrometry is not sufficiently sensitive. Nevertheless, this situation may alter in the near future, when mass spectrometry with its better discrimination will likely be deemed to be essential. Our group has applied matrix-assisted laser desorption time-of-flight mass spectrometry for analysis of tryptic digests of hCG [28]. More recently, Bowers et al. have used electrospray ionization high-performance liquid chromatography-mass spectrometry (HPLC/MS) to identify tryptic fragments of hCG corresponding to 25 IU/L of holo-hCG in 10 mL of urine [29, 30]. They anticipate that with improvements in the entire sample-cleanup procedure they can improve the limit of detection to the 10 IU/L suggested by Laidler et al. [31]. Because of differences in mass of selected tryptic fragments from other peptide hormones, such as human luteinizing hormone, these soft-ionization methods appear to offer great specificity. Soft-ionization procedures also show potential for detecting other peptide hormones used as doping agents. For example, administration of somatotropin to healthy men results in a large increase in plasma insulin-like growth factor-I (IGF-I) as measured by RIA [32]. Bowers reports detection of 10 fmol of IGF-I by HPLC/MS [5].

Development in mass spectrometry also has opened up avenues to enhance the detection of anabolic steroids in urine of sports competitors. Bowers compares the use of high-resolution mass spectrometry using the lower-cost approach with quadrupole ion traps in the tandem mass spectrometry mode; he also mentions considerations on using an ion trap of the earlier type, which had an internal ion source, or the more modern ion trap with external ionization. When using the mass spectrometer, or indeed any other measuring device, one is trying to enhance the
signal produced by the analyte of interest from the surrounding noise. So-called “high-resolution” capillary gas chromatography has done much to separate the biological noise background from the individual analytes to be determined. Significant sample clean-up is facilitated by the appropriate use of immunoaffinity chromatography. Interestingly, laboratories using high-resolution mass spectrometry to screen samples for the presence of anabolic steroids and their appropriate longer-lived metabolites tend to confirm their findings by normal resolution in full-scan mode, having first concentrated the urine sample by immunoaffinity chromatography. The stability of the spectra produced by tandem mass spectrometry has been well debated, and the absence of MS-MS libraries suggests that a number of problems still need to be resolved. In our hands, we find that the spectra obtained by MS-MS for an authentic reference material compare well with those obtained from the sample under investigation when run in the same assay.

Although only the scientific presentations are included in the published proceedings, the presentation by a lawyer, Mark Gay of Messrs. Herbert Smith, is worthy of mention. A large part of the symposium was devoted to detecting the presence of doping agents in a sample from a sports competitor, but without careful consideration of several surrounding issues, the laboratory data are valueless. Even before the laboratory receives a sample, the issue of consent must be resolved. Gay asked how we can justify violating an individual’s privacy to take a blood or urine sample, and how far can a sport legitimately go and, indeed, must go, in seeking to catch cheats. From his experience of working for a large international sports federation, he concluded that, provided the rules have been carefully considered and properly constructed and are then published, the courts have permitted sport to collect samples for drug-control purposes. In the clinical chemistry context, the issue of a laboratory’s legal or ethical duties in circumstances where it discovers that the athlete may have a pathological condition is clear. However, an apparent conflict remains, between “patient” confidentiality and reporting a finding that may have clinical significance, not to the donor of the sample, but to a third party. The answer to this dilemma must be to think about it before it happens and to set up a proper mechanism to ensure that the information is received by the individual concerned or his or her physician in an approved manner. Finally, Gay discussed how sports competitors challenge the laboratories, in seeking to invalidate the results of a positive test. This raises the issue of publicizing our work. Some say we should not publicize any weaknesses in an analytical system. Clearly, not only is this unscientific but such a short-term expediency leads to charges of secrecy and in the long term is doomed to failure: The exposure of the secrets of East German system may be cited in this context. However, much time is wasted to legal challenges based on poor-quality evidence that has been published. Surely, the only answer is for our work to be aired and challenged by publication in good-quality peer-reviewed journals. We hope that the proceedings of this symposium will fit that category.

This editorial is dedicated to Raymond Brooks, a truly remarkable scientist, who has accomplished so much in the field of anti-doping in sport; we thank him for his continued support in our work.

References


David A. Cowan
Andrew T. Kicman
Drug Control Centre
King’s College London
Manresa Rd.
London SW3 6LX, UK
Fax +44-171-351-2591
e-mail da.cowan@kcl.ac.uk
a.kicman@kcl.ac.uk