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Testing for Drug Abuse

David A. Cowan

Drug Control Centre, King's College London, Manresa Road, London SW3 6LX, United Kingdom

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1. INTRODUCTION

Drug abuse in sport is commonly known as doping. The International Olympic Committee (IOC) uses the term "doping" to describe substances whose use is prohibited in sport. In addition, certain so-called "methods" are also prohibited. The IOC state that doping contravenes the ethics of both sport and medical science. Most international federations follow the lead given by the IOC in formulating their doping rules. Figure 1 shows a list of the different categories of substances and methods prohibited by the IOC. In this paper, several current issues relating to the laboratory analysis of samples collected as part of drug control programmes will be discussed.

INTERNATIONAL OLYMPIC COMMITTEE - MEDICAL COMMISSION

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C. Anabolic Agents
D. Diuretics
E. Peptide and glycoprotein hormones and analogues

II. PROHIBITED METHODS

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A. Alcohol
B. Marijuana
C. Local anaesthetics
D. Corticosteroids
E. Beta-blockers

Figure 1. Prohibited classes of substances and prohibited methods - January 1996

2. PROHIBITED CLASSES OF SUBSTANCES AND PROHIBITED METHODS

The laboratory analysis of samples collected from sports competitors is focused mainly on detecting the prohibited classes of substances. These classes are subdivided into five groups. The first of these classes is the stimulant class (Figure 2). This class includes drugs such as amphetamine and cocaine as well as milder stimulants such as ephedrine. Several of these milder stimulants are available without a medical prescription from pharmacies in many countries for the symptomatic treatment of colds.

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I. PROHIBITED CLASSES OF SUBSTANCES

A. Stimulants

Prohibited substances in class (A) include the following examples:

amiphenazole	amphetamines
amineptine	caffeine *
cocaine	ephedrines
fencamfamin	mesocarb
pentylenetetrazol	pipradrol
salbutamol **	terbutaline **
salmeterol **	

... and related substances

* For caffeine the definition of a positive depends on the concentration of caffeine in the urine. The concentration in urine may not exceed 12 micrograms per millilitre.

** Permitted by inhaler only and must be declared in writing, prior to the competition, to the relevant medical authority.

Figure 2. Prohibited classes of substances - stimulants

Although the mere presence of most substances shall constitute, in the words of the IOC, "a definitive case of doping" for some there is a quantitative limit which is permitted. In the stimulant category, this is the case for caffeine where the concentration in urine may not exceed 12 micrograms per millilitre. The rules are written so as to create an absolute offence since it is very difficult to prove intent and impossible to do so solely from the analysis of a single urine sample which currently is the only body fluid routinely collected.

The IOC ban classes of substances and, under their rules, simply give a list of examples. The list given in most of the classes of prohibited substances concludes with the phrase "and related substances". This construction of the rules prevents the use of so-called designer drugs from circumventing the rules but may cause some debate about what is prohibited and what is permitted. This is exactly what happened during the 1996 Olympic Games held in Atlanta. The laboratory identified the presence of bromantan metabolites in the urines of several Russian competitors. The IOC considered bromantan a stimulant and disqualified these competitors. Two of the competitors took their cases to the Court of Arbitration for Sport. The court decided that their appeal should be upheld because it considered that there was insufficient evidence put before the court to make such a conclusion. In the UK, The Sports

Council provides an information service which includes a so-called "hot-line" telephone service, to assist competitors in interpreting the rules.

3. SYNTHETIC AND ENDOGENOUS ANABOLIC STEROIDS

The anabolic agents class is subdivided into the anabolic androgenic steroids (AAS) and beta-2 agonists (Figure 3). The AAS sub-class consists of testosterone which is the natural androgen produced endogenously in males and also, but to a lesser extent, in females and synthetic modifications of this steroid.

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I. PROHIBITED CLASSES OF SUBSTANCES

C. Anabolic agents

The Anabolic class includes anabolic androgenic steroids (AAS) and Beta-2 agonists.

Prohibited substances in class (C) include the following examples:

1. Anabolic androgenic steroids (AAS)

clostebol
metandienone
nandrolone
stanozolol
... and related substances

fluoxymesterone
metenolone
oxandrolone
testosterone

2. Beta-2 agonists

clenbuterol
salbutamol
terbutaline
salmeterol
fenoterol
... and related substances

Figure 3. Prohibited classes of substances - anabolic agents

The synthetic AAS are routinely detected using capillary column gas chromatographs linked to low resolution "bench-top" mass spectrometers operating in the selected ion monitoring (SIM) mode. The resolution of the capillary column together with the selectivity of the mass spectrometer operating in the SIM mode provides a relatively sensitive method of analysis being able to detect concentrations of as little as 10 ng/ml in a 2-5 ml sample. Samples which fail the SIM screen are then subjected to confirmatory analysis using full scan mass spectrometry. Earlier this year, the IOC announced that high resolution mass spectrometry (HRMS) would be used at the Olympic Games in Atlanta. The benefit of HRMS is that the biological (noise) background is further reduced by the additional selectivity provided by HRMS. This enhances the signal to noise ratio and improves the detectability of substances approximately ten-fold. The confirmatory procedure still makes use of full scan mass spectrometry. Since the approximate concentration of the substance to be confirmed can be determined from the screen, the confirmatory procedure can be suitably modified by use of a larger than normal volume of urine which is concentrated using immunoaffinity in order to obtain the definitive full scan mass spectrum. Currently, this technique has been developed for a relatively small number of synthetic anabolic steroids and, unfortunately, the HRMS instrument is relatively expensive. An alternative approach is to use the technique known as

MS-MS. In this mass spectrometric technique, the mass spectrometer is set up to exclude all ions except for the chosen one or several. These ions, known as precursor ions, are then allowed to fragment. The spectrum of the resulting fragment ions may be obtained, or simply one or a few fragment ions selectively measured. This technique gives very good discrimination from possible interferants and also good sensitivity. The technique is available on a variety of the more sophisticated, and expensive, mass spectrometers. A low cost version is now also available using ion trap technology. The use of this low cost instrument is currently being investigated by several IOC-accredited laboratories world-wide to determine whether this may provide a more cost-effective approach to achieve the required enhanced sensitivity.

Although the mere presence of a synthetic AAS in a test sample constitutes a contravention of the rules, since testosterone is produced endogenously, a threshold based on testosterone to its natural, but apparently inactive, related compound epitestosterone is used. Figure 4 shows the IOC's current ruling on testosterone.

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TESTOSTERONE

The presence of a testosterone (T) to epitestosterone (E) ratio greater than six (6) to one (1) in the urine of a competitor constitutes an offence unless there is evidence that this ratio is due to a physiological or pathological condition, e.g. low epitestosterone excretion, androgen production of tumour, enzyme deficiencies.

In the case of T/E higher than 6, it is mandatory that the responsible authority conduct an investigation before the sample is declared positive. A full report will be written and will include a review of previous tests, subsequent tests and any results of endocrine investigations. In the event that previous tests are not available, the athlete should be tested unannounced at least once per month for three months. The results of these investigations should be included in the report. Failure to cooperate in the investigations will result in declaring the sample positive.

Figure 4. International Olympic Committee ruling on testosterone

Since testosterone is usually administered as an ester, e.g. oenanthate, propionate, phenylpropionate or undecanoate, the finding of the intact ester would indicate that testosterone had been administered. Unfortunately, no intact ester of testosterone has ever been reported to be found in the urine. However, work by de la Torre and co-workers [1] has shown that intact testosterone esters may be found in blood samples post administration. At present however, since only urine samples are routinely collected, this approach does not have current applicability.

In practice, evaluation of results of tests obtained previous and subsequent to the finding of an elevated testosterone to epitestosterone ratio is the main approach to determining whether the abnormal finding was most likely the result of testosterone administration. The administration of ketoconazole is a useful tool to distinguish the biological outlier from the cheat. Ketoconazole blocks 17α-hydroxylase/17,20-lyase activity thereby inhibiting the endogenous synthesis of testosterone. This method has been described by Kicman and co-workers [2] and the method refined by Oftebro and co-workers [3].

An alternative approach still under development is the use of the $^{12}C:^{13}C$ ratio [4]. This method relies on the fact that administered testosterone is manufactured from a synthetic

steroid usually obtained from a plant precursor which has a ^{12}C:^{13}C ratio which is different from that of the endogenously biosynthesised testosterone. It is questionable as to whether this approach will be of much value in dealing with the more difficult cases especially those with a testosterone:epitestosterone ratio near to six caused by a small concentration of epitestosterone rather than a large testosterone concentration.

Unfortunately, most of the currently used methods for detecting testosterone administration may be circumvented by the co-administration of epitestosterone as shown by Kicman and co-workers [5]. These authors have shown that the use of the ratio of testosterone to luteinising hormone cannot readily be circumvented. The IOC endeavour to deal with this possibility by prohibiting "epitestosterone application" as stated in Article II of their rules (Figure 5). However, the current rules give no guidance as to what will be accepted as evidence of manipulation. This rule also bans the use of probenecid which was probably the most important masking agent. Probenecid acts by inhibiting the elimination in the urine of many anabolic steroids by blocking active secretion in the kidney tubules.

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Article II : PROHIBITED METHODS

The following procedures are prohibited:

Blood doping

Blood doping is the administration of blood, red blood cells and related blood products to an athlete. This procedure may be preceded by withdrawal of blood from the athlete who continues to train in this blood depleted state.

Pharmaceutical, chemical and physical manipulation

Pharmacological, chemical and physical manipulation is the use of substances and of methods which alter, attempt to alter or may reasonably be expected to alter the integrity and validity of urine samples used in doping controls, including, without limitation, catheterisation, urine substitution and/or tampering, inhibition of renal excretion such as by probenecid and related compounds and epitestosterone application.

The success or failure of the use of a prohibited substance or method is not material. It is sufficient that the said substance or procedure was used or attempted for the infraction to be considered as consummated

Figure 5. Prohibited methods

4. PEPTIDE HORMONES

All of the above mentioned classes are tested for by a variety of screening techniques. Should the presence of prohibited substances be suspected from the screen, they are identified and confirmed using mass spectrometry. The mass spectrometer is a rather expensive and sophisticated instrument which requires relatively skilled scientists to use it reliably. The competence of the scientists is tested by the IOC in its accreditation programme. The detection of the peptide hormones (Figure 6) requires a different approach since they cannot, at present,

be analysed using mass spectrometry. Instead immunoprocedures must be used. These may be very selective or specific but a single procedure lacks the discriminating power of mass spectrometry. Work is underway to develop mass spectrometric methods for these substances but probably this will not be successful for a number of years. In the meantime, the IOC requires the use of more than one validated immunoprocedure before declaring a finding of hCG. This is the only substance in this class for which currently there is an accepted procedure.

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E. Peptide and glycoprotein hormones and analogues

Prohibited substances in class (E) include the following examples:

1. Chorionic Gonadotrophin (HCG - human chorionic gonadotrophin)

2. Corticotrophin (ACTH)

3. Growth hormone (HGH, somatotrophin)
... and all the respective releasing factors for such substances.

4. Erythropoietin (EPO)

Figure 6. Prohibited classes of substances - Peptide and glycoprotein hormones and analogues

Nevertheless, work is well advanced on developing a test to be able to detect erythropoietin administration [6]. This test is based on the large changes in the serum soluble transferrin receptor content following erythropoietin administration and relates this to serum ferritin, a measure of body iron store. Similarly, a large project began last year to develop a test to detect growth hormone administration in time for the Olympic Games to be held in Sydney, Australia, in 2000. The first publications on possible methods to detect GH administration are already starting to appear and that by Kicman and co-workers [7] looks at the use of the binding proteins as well as the insulin-like growth factors. However, the methods under development to detect EPO and GH administration all appear to require the use of blood samples.

5. EFFECTIVENESS OF LABORATORY ANALYSIS

The IOC-accredited laboratories usually receive pairs of selected samples which are individually coded so as not to reveal the identity of the competitor. One of the pair usually coded with the prefix A is opened and analysed. Should this sample reveal the presence of a prohibited substance the governing body is informed. If required, the seal of the second of the pair coded with the prefix B is analysed in the presence of witnesses including the competitor if he or she wishes. This system, together with the use of highly specific and discriminating analytical procedures is used to avoid a competitor being falsely accused of having taken a banned drug.

The IOC monitors the drug testing programmes in which its 24 accredited laboratories world-wide are involved in a number of ways. Analytical findings are collected and published

by the IOC. Figure 7 shows a summary of annual findings for the years 1986 to 1994. The open columns indicate the number of samples analysed and has shown a steady increase from about 33,000 in 1986 to 95,000 in 1994. The proportion of analytically positive samples is indicated by the shaded columns and fluctuates with a maximum of nearly 2.5% in 1988 and a minimum of 1% in 1991.

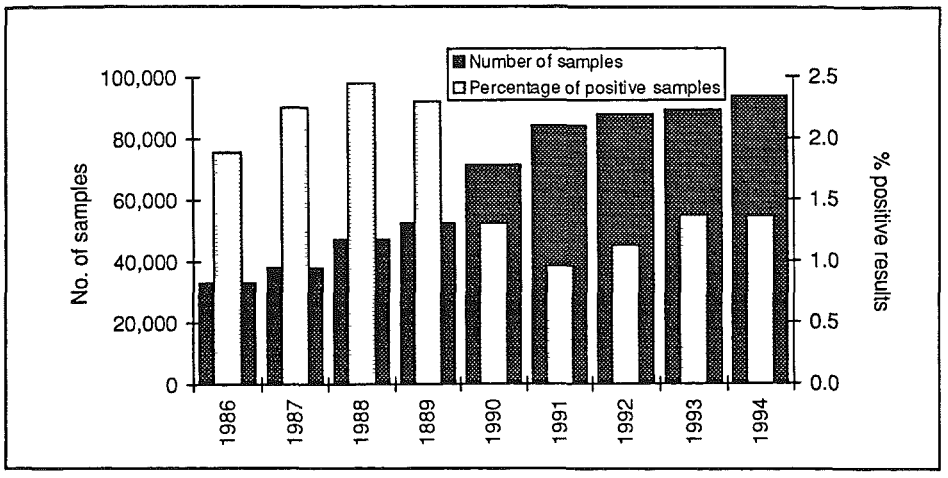

Figure 7. IOC Accredited Laboratories Summary of Samples Analysed

Figure 8 shows a summary by category of the identified substances for the years 1992-1994 from which it may be seen that the anabolic agents are consistently the commonest finding. In 1994 testosterone cases were the commonest followed by nandrolone. The finding of ephedrine in the stimulant class accounted for nearly as many cases as for nandrolone.

IOC Summary of Identified Substances by Category

IOC CATEGORY	1994	%	1993	%	1992	%
A. Stimulants	347	27.2	331	27.1	277	22.1
B. Narcotics	42	3.3	46	3.8	102	8.2
C. Anabolic Agents	891	69.7	940	76.9	717	57.3
D. Beta-blockers	15	1.2	11	0.9	12	1.0
E. Diuretics	63	4.9	65	5.3	70	5.6
Masking agents	8	0.6	7	0.6	22	1.8
Peptide hormones	3	0.2	4	0.3	4	0.3
Others	77	6.0	48	3.9	39	3.1
TOTAL	1278		1222		1251	

Some samples contain more than one substance from the prohibited classes.

Figure 8. IOC summary of identified substances by category

These findings should not be taken to be representative of the level of misuse which, unfortunately, is probably considerably higher. Some substances may be detected at lower concentrations and some substances are taken at larger doses than others. Thus it is not possible from these figures alone to determine the relative distribution of which substances are taken. Nevertheless, since effective drug control programmes were introduced, at least the deaths from amphetamine misuse have ceased.

6. CONCLUDING REMARKS

In case the competitors are getting better at evading the tests, the IOC announced that high resolution mass spectrometry would be used this year at the Olympic Games in Atlanta. This type of mass spectrometry is at least one order of magnitude more sensitive than for the normal resolution mass spectrometry previously routinely used by the IOC accredited laboratories. Its sensitivity is achieved mainly by removing the effect of interference from the sample being analysed. Unfortunately, the instruments are extremely expensive and require highly trained scientists to operate them effectively.

Clearly, if drug control in sport is to succeed effective laboratory analysis is one element that must not fail. Since the whole field is currently advancing, strong research and development programmes are vital. We at King's College intend to continue our work to assist in the control of drug abuse in sport.

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Discussion: Testing for Drug Abuse

M. Gleeson:
Could you comment on the possibility of the modification of metabolites of testosterone via bacterial contamination of urine as was implied in the Diane Modell case?

D.A. Cowan:
The laboratory responsibility begins when the sample comes through their door. But they also have a scientific, ethical responsibility. If they see something wrong with a sample, they have to say so. The sample of Mrs. Diane Modell reached the laboratory some two days after the time of collection. Then there was a question as to whether the sample had degraded during that transit to the laboratory. The possibility of interconversion between the steroids is pretty well known anyway. Some artifacts we may generate because of the hydrolysis process we conduct. Because urine is not necessarily sterile, the possibility of microorganisms growing in the urine sample and causing interconversion is something else that we do have to take note of.

B. Ekblom:
The testing with ketoconazole introduces a new type of detection principle. Can take back an athlete and say, can we test you and see if your T:E ratio is going down with this drug? Do you think that this will be possible in the future? That all the possible positive T:E tests will be retested in these subjects?

D.A. Cowan:
The International Olympic Committee already requires multiple measurements now on T:E cases. Either from previous data or from data collected after that first finding. They say at least three measurements. I am uncomfortable on the ethical basis of saying to an athlete, "You must take this drug to prove your innocence" and we do not suggest that. But what we do say is, to the athlete, "This is one of the tools that is available. If you feel that would be useful and you wish to undergo the test, with proper informed consent, then here it is. This is to help you, not to force you."

D.P.M. MacLaren:
In our university we were involved in doing some of the testing in support of Diane Modhal's case. We had a number of female athletes who were both creatine-loaded or not creatine-loaded. Diane Modhal was creatine-loaded at the time when she was supposedly tested positively. When the urine samples were stored under different conditions (kept in the refrigerator early on or left standing at room temperature) we did find significant changes in the testosterone : epitestosterone ratio which seemed to support the contention that storage of urine samples may prove problematical in these instances, i.e. steroid transformation occurs at room temperature but not likely under refrigeration.

D.A. Cowan:
Just a comment back on that. In the tests that we have conducted, the majority tended to show a drop in the testosterone : epitestosterone ratio. One needs to be careful because if you

drop epitestosterone more than testosterone, the ratio would apparently increase. So that this is one of the very big difficulties of the whole issue. What was interesting in her case was she had a relatively large testosterone concentration as well. And so we are looking also at a possible increase in production of testosterone from other hormones. Her specific case has got just to continue as a mystery. And it is unfortunate, because those sorts of doubts are never comfortable in the evidential sense. It has put a slur on her name, whether she is innocent or guilty. That slur will never disappear. It also puts a slur on the testing system.

One of the points I made when I first came in on the case is how little is published about testosterone administration in females. A lot has been done in males, but we know very little among females. It is interesting to note that when testosterone is being used for the treatment of premenstrual syndrome, just the oral administration of testosterone can also produce a highly raised testosterone:epitestosterone ratio.

T.D. Fahey:

I have a question concerning dehydroepiandrosterone (DHEA). It has become very popular with athletes in the United States, but it is a naturally occurring substance. Could you comment on testing for this substance?

D.A. Cowan:

DHEA, as you know, is a mild androgen. At the present time, we take the view that DHEA is not a banned substance unless it starts to change things like the T:E ratio. That is the way the rules are constructed. Note: In November 1996 the IOC took the view that DHEA is a related substance in the anabolic agent category.

A.J.M. Wagenmakers:

I have a question about the use of tracers in drug testing. You already mentioned that you can use the ^{13}C-enrichment of anabolic steroids to discriminate between steroids synthesised endogenously and those taken as a supplement from a pharmaceutical source. It probably is a naive question, but let me ask it anyway: Cannot we approach pharmaceutical companies to use stable isotope labelled tracers during the synthesis of drugs? The high tracer enrichments that will be obtained in that case will make drug testing into an easy task for the analytical laboratories and highly discriminative. I realise that there are immense commercial interests and many potential escape routes, but should not we consider this approach anyway?

D.A. Cowan:

It is an issue that we have considered many times, but the pharmaceutical companies are forced to deliver extremely pure products, even sometimes to the level of enantiomeric purity. The philosophy, then, of artificially changing it is problematic.

D.R. Mottram:

We are now talking about asking competitors to take drugs like ketoconazole which is a therapeutic drug, for a non-therapeutic purpose. We have also got the possibility of blood sampling which is an invasive process and of course runs the risk of possible infection. So what are the chances of litigation by competitors in the future who are asked to do this sort of procedure as part of the drug testing process? Where are we going with this?

D.A. Cowan:

It was one of our concerns when we were developing the ketoconazole test that we did not want to force athletes to take the test, but recognising at the same time that the moment you had that facility, you might well get in the arena where we are saying, "Well, look if you are innocent, take it." The ethical side we do have to think through very carefully. I advise to try to avoid giving athletes drugs and using any of the more invasive techniques in order to prove their innocence.