MISUSE OF DRUGS IN SPORT

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HISTORICAL BACKGROUND

The misuse of drugs in sport is not a new phenomenon. It has occurred for centuries but adequate documentation is only available for the last 50 years. It was the deaths in sport because of misuse of drugs and also the increasing problem of drug misuse in society which called into play national and international reactions to deal with the problem. It has been stated that in the few years before 1970 there were 30 deaths in sport through drug misuse. An anti-doping law was passed by the French Senate and became effective in 1965 and in the same year Belgium introduced an anti-doping act to deal with the problem of drug misuse in sport.

The International Olympic Committee Medical Commission was established in 1967 and dope control was one of its main responsibilities. The death of the Danish cyclist Jensen in the 1960 Olympic Games in Rome and the circulating stories of drug misuse at the Olympic Games in Tokyo in 1964 led to pressure for international action.

We at Chelsea College were brought into the sphere of dope control in sport almost by accident. From about 1958 we had been carrying out work on drug distribution and metabolism and elimination in man (Beckett, 1966 and Beckett and Tucker, 1967). We were concentrating especially in the field of sympathomimetic amines and narcotic drugs. This work necessitated the development of analytical techniques for determining very small amounts of drugs and metabolites in biological fluids.

We presented some of our earlier work, in which we chose amphetamines and related compounds as our examples, to an International Research Symposium on "Medicinal Chemistry" at Chelsea College in the Easter of 1965. During the Conference a friend of A.H.B. from Belgium expressed interest in the work and said that obviously we now had sensitive methods of analysis for drugs such as amphetamines. He further asked for how long we could detect amphetamines in man after an oral dose had been given. When told about 48 hours he said that A.H.B. should notify sports authorities of our important sensitive methods because in many countries a very serious problem of drug misuse in sport was developing.

Some sports authorities in Europe had organised dope control tests, and urine from competitors was being

checked, but results from laboratories were reported as negative even when it was known generally that competitors being tested were using drugs. In other words tests being used were not sufficiently sensitive.

Our work has centred around the use of gas chromatography for determining these amines in biological fluids. Also we established the various conditions which lead to different amounts of drugs in urine as circumstances changed. A few examples will serve to give the basis for this work.

Gas chromatography can be used to separate quite closely related amines (Fig. 1). A single peak, for instance of amphetamine, can be converted readily by use of reagents into a variety of peaks with very different retention times (Fig. 2). It is also possible to make use of a knowledge of metabolism of the drug to establish that the drug has been given to man. For instance if methylamphetamine is given then one must find both the drug and its metabolite, amphetamine, in urine. Figure 3 shows the results from a positive result from a racing cyclist's urine.

The development of analytical methods alone is not sufficient to ensure good dope control. A knowledge of the application of the methods to the biological system is necessary and a knowledge of what happens to drugs under different conditions is also required. For instance, after a basic drug is given to man there is not a smooth rate of excretion of drug against time; there are fluctuations which are controlled by urinary pH (Fig. 4). This means that a competitor could have ingested a large amount of a basic drug and yet if the urine is alkaline there would be very little of the drug to be measured in the urine (Fig. 5). Thus very sensitive methods would be required under conditions of alkaline urine.

As these techniques were developing so was the more definitive analysis by mass spectrometry; this will be described in more detail later. The newly devised tests (Beckett et al 1967) were first used in the dope control in the UK for sports involving humans in 1965 in the Tour of Britain, the so called Milk Race. The results led to three disqualifications and thus the new methods were beginning to have an effect.

The next use of the methods on a big scale was the World Cup in the UK in 1966 (Beckett et al 1966). This was the first time there had been dope control in soccer at international level. There were no disqualifications in

Fig. 1: Composite chromatogram of some stimulants and related compounds on column "A".

this World Cup but the sensitivity of the test was in evidence because we were determining very small amounts of bases in urine and it was established subsequently that these had arisen from the use of certain nasal drops by some competitors.

In the early days of the tests, many of the sports writers were not in agreement. They complained that the tests were interfering with the rights of individuals. They were not convinced that the tests were fair and gave correct results. They had experience of what was happening on the Continent and therefore they had quite legitimate doubts. However, within a few years the same sports writers were conceding that many of the athletes were in favour of the tests. They had seen what was happening internationally to top competitors and they had seen the increased compulsion for drug taking to achieve success. Many competitors in the amateur ranks therefore wanted to curtail the escalating drug

misuse in sport. Once these sports writers had seen that the newer tests were fair and competent and the whole scheme of sampling and control was effective, they became strongly in support of the work and in fact began to criticise that no action was being taken in the field of anabolic steroids.

In the early days the question was sometimes asked "why should not the right of choice be allowed to any competitor to use any method of training or even any drug despite possible danger to health or life. If he dies in the attempt to excel is that not his responsibility?"

Some people have stated that the use of a drug contravenes the basic characteristics of sport. In other words they have argued solely on moral grounds that sport should be the matching of skills of the individuals and not boosted artificially. However, there are other compelling arguments because unfortunately many

Fig. 2: Composite chromatogram of amphetamine and some of its derivatives on column "C".

competitors have not got the right of choice. Sometimes physicians and coaches do not put the welfare of those under their care first in their thoughts. Surely our young people must be protected against the unscrupulous physician or coach who would use the competitor as a guinea pig and would use potent drugs upon him irrespective of potential consequences? Also the use of some drugs can cause aggression and loss of judgement, for instance the use of amphetamines or anabolic steroids in a competitor can constitute a hazard to other competitors or spectators who are not involved in drug

misuse. Furthermore we have a major problem of drug misuse in society and many young people are involved. If it were established that many of the leaders in sport, the heroes and heroines of the young people, were misusing drugs to achieve performance then there would be devastating effects on young people as a whole.

The International Olympic Committee Medical Commission addressed itself to the problem of drug misuse in sport and attempted to produce plans and guidelines. The first introduction of its plans on a tentative basis

Fig. 3: Reproduction of a chromatogram obtained on analysis of a racing cyclist's urine showing the presence of methylamphetamine and its metabolite amphetamine in the urine.

was in the Olympic Games in Grenoble in 1968 and a much more comprehensive scheme based upon this experience was introduced into the Olympic Games in Mexico in 1968.

The policy of the Commission was to attempt to prevent the misuse of drugs in sport which constituted dangers when used as doping agents and to deal with this control with the minimum of interference with the correct therapeutic use of drugs. It was accepted, for instance, that some competitors would have colds at international events and would need treatment. Some would have difficulty in going to sleep after travelling long distances and would require sedation. Therefore the plan was to deal with the problems but still allow adequate and correct therapeutic treatment.

Another point uppermost in the mind of the Commission was only to ban those compounds for which suitable analytical methods could be devised to detect

Fig. 4: The influence of urinary pH and urine output on the urinary excretion of amphetamine in man, after oral administration of 15mg (+)-amphetamine sulphate (Similar patterns were observed in other subjects).

Fig. 5: Cumulative urinary excretion of methylamphetamine in man under varying conditions of urinary pH after oral administration of 1.0mg (+)-methyl-amphetamine. (Subject E.J.T.).

the compounds unequivocally. In other words objective methods of assessment were to be used and denunciations were not to be accepted as a basis for action. This is why although there was a widespread misuse of anabolic steroids in 1967, these compounds were not banned since tests were not available.

TABLE I

**Drugs banned by the International Olympic Committee
– Medical Commission, 1968.**

- a) **Psychomotor stimulant drugs, e.g.**
 - amphetamine
 - benzamphetamine
 - cocaine
 - diethylpropion
 - dimethylamphetamine
 - ethylamphetamine
 - fencamfamin
 - methylamphetamine
 - methylphenidate
 - norpseudoephedrine
 - phendimetrazine
 - prolintane
 - and related compounds
- b) **Sympathomimetic amines, e.g.**
 - ephedrine
 - methylephedrine
 - methoxyphenamine
 - and related compounds
- c) **Miscellaneous central nervous system stimulants, e.g.**
 - amiphenazole
 - bemegride
 - leptazol
 - nikethamide
 - strychnine
 - and related compounds
- d) **Narcotic analgesics, e.g.**
 - heroin
 - morphine
 - methadone
 - dextramorphamide
 - dipipanone
 - pethidine
 - and related compounds

Another problem immediately became apparent, namely that to attempt to try and draw up a definitive list of banned drugs would not solve the problem. It was known for instance that compounds with similar activity to materials we would like to ban could be used to circumvent the ban. For instance in society the

compound STP was being misused in the USA especially, and had not been marketed as a drug. These considerations therefore led to an approach which listed banned classes of drugs and gave examples of some of the compounds in the banned classes but did not restrict itself to these examples only, the statement 'related compounds' was included after the examples in each section. The list of doping classes used in the 1972 Olympic Games in Munich illustrates the outcome of this general philosophy (see Booklet "Doping", 1972).

Work was continuing in an attempt to produce a suitable test for anabolic steroids. Professor Raymond Brooks of St. Thomas Hospital with a grant from the Sports Council was working on the development of radioimmunoassays to deal with the problem. The work became sufficiently advanced so that the I.O.C. Medical Commission was able to include anabolic steroids amongst the banned classes in April, 1975 as follows:

Anabolic steroids e.g.
methandienone
stanozolol
oxymetholone
nandrolone decanoate
nandrolone phenylpropionate
and related compounds

When this decision was made, it was known that the work on the glc/mass spectrometry to establish each particular anabolic steroid in urine was reaching fruition. Thus the decision in 1975 allowed action to be taken at the 1976 Olympic Games in Montreal. In Montreal, therefore, the radioimmunoassay on anabolic steroids was used as a screening test and those samples which gave an apparent positive result were then carried through for detailed examination by glc/ms. The radioimmunoassay test produces some false positives especially in women in which some of the components of contraceptive pills give a positive result in the radioimmunoassay for anabolic steroids. However, the screening test meant that many samples could be established as negative so that a few samples needed to be taken through to the more demanding glc/ms work, i.e. the test which gave the unequivocal identification of the anabolic steroid used.

COLLECTION AND CONTROL OF SAMPLES

The taking of samples and the control of samples is just as important as the final analysis. Some of the points recorded in the final dope control booklet issued for the Games in Montreal will illustrate this point.

"Immediately following the taking of the sample, the competitor shall select another sealed bag which contains two bottles. He shall himself pour an equal amount of urine in each bottle and shall close them securely.

The official in charge of the station shall check the pH of the urine. If the pH is not satisfactory, the procedure described in 4.2.13 shall be followed.

The official in charge of the station after verifying that the two bottles are well closed, shall codify them with a control number selected by the competitor and shall seal them.

The official in charge of the station shall give the competitor and the accompanying person an opportunity to make sure that the bottles are correctly sealed.

The code number shall be noted in the records by the official in charge of the station. The medical technician shall give the competitor and the accompanying person an opportunity to ascertain that the number noted in the records agrees with that recorded on the two bottles.

The competitor shall certify by signing the records that there have been no irregularities in the entire sample-taking procedure. The records shall also be signed by the official in charge of the station and the accompanying person (if any), and shall be placed in separate envelopes and sealed.

The envelope containing the original copy of the records shall be sent through the chairman of the Doping Control Committee, to the chairman of the IOC Medical Commission. For security reasons the duplicate copy shall be kept sealed, in a safe, until the end of the Games.

The medical technician shall place each of the bottles in a container which shall then be sealed immediately.

All the sealed containers, each holding a sealed bottle, shall be placed in a special box which itself shall be sealed before being transported to the laboratory.

This sealed box shall be given to the courier upon signature of a receipt which will indicate the number of samples in the box, the site from which they come and the departure time of the courier.

The courier shall take the sealed box to the laboratory immediately.

At the laboratory, a person appointed by the head of the laboratory shall acknowledge receipt of the sealed boxes. This person shall take note of the time of arrival."

If a positive result is obtained on the first analysis, then the duplicate sample is analysed in front of witnesses. For instance the following rule applied in Montreal,

"The analysis of the duplicate sample shall be carried out in the same laboratory but by different persons. The analysis shall be supervised by a member of the IOC Medical Commission. The delegation in question shall be allowed for instance

a maximum of three (3) representatives to the laboratory."

Effective doping control requires well equipped laboratories and highly skilled staff. A laboratory without the necessary experience can report a negative result on samples which in fact contain drugs and therefore it is important that authorities in sport only use laboratories which have been approved for those specific purposes. Problems have occurred in international events when non-approved laboratories have been used for the repeat analysis on the duplicate sample. It is important in dope control procedures to have samples of urine in which known drugs are present, and the bottles containing these samples are coded in the normal way as if they were from competitors. Frequently volunteers amongst members of the Medical Commissions of the various sports take drugs on request and their urine is collected and treated in this manner. The identification of the drug collected by the laboratory establishes not only the competence of the laboratory but the whole method of operation of dope control.

The organisation of dope control at the Olympic Games is a big undertaking. The problem is that many samples have to be analysed and the results have to be obtained quickly. These numbers and the limited time allowed between the receipt of samples and the report of the results creates many problems not usually encountered in operations on a smaller scale. For instance at the Munich Olympic Games 2079 urine samples were screened for the presence of drugs and of course at this time there were not any tests for anabolic steroids. In Montreal at the 1976 Olympic Games, 1,800 urine sample were tested in the conventional dope control and 275 samples in the anabolic steroid controls. The international federations co-operate with the IOC Medical Commission and many federations accepted the general rules laid down by the Medical Commission. Thus slowly, over the years, systems have developed although at present not all federations deal with dope control in an adequate manner.

The function of dope control in sport is to constitute a deterrent, rather than to be punitive in its approach. Some people remark that there are few people caught in dope control and therefore question the need for such control. For instance they quote that of the 2079 urine samples collected in Munich only 9 positive results were obtained and of these 9, 7 results led to disqualification. At Montreal there were three positives in the conventional dope control and eight positive results in the anabolic steroid control.

ADVERSE EFFECTS AND DANGERS FROM DRUG MISUSE IN SPORT

As already indicated, there have been deaths in sport

through the use of stimulant drugs. Also the continued use of some drugs can cause psychological and physical harm to those who take them. For example amphetamines can produce psychoses and psychological dependence and narcotics in addition can produce physical dependence.

The following has been written about the problem arising from the misuse of anabolic steroids. ("Doping", 1972).

"There are very few adverse reactions to normal treatment with anabolic steroids, but misuse may lead to pathological disturbances which vary according to the ratio of androgenic/anabolic activity in the compounds used. Some products cause gastro-intestinal disturbances, liver dysfunction, cholestatic jaundice, increase in blood pressure secondary to water retention, and acne. The inhibition of the physiological pituitary testicular feedback mechanism may cause decrease in spermatogenesis and testicular shrinkage. Prostatic troubles due to the hypertrophy of this gland can also occur.

In females, menstrual troubles and masculinization symptoms, such as hirsutism and deepening of the voice, may occur. The premature sealing of the epiphyseal plates of long bones may stop growth in children and adolescents. Another danger also reported in attempting to maximise muscular volume and efficiency by the use of anabolic steroids is that tendon strength is not increased in proportion to the increase in muscle mass. Since certain groups of muscles, particularly biceps and quadriceps, are submitted to constantly increasing strain, such conditions as inflammation of the knee tendons, and the partial or total rupture of long muscle tendons may occur. The skeleton is also subjected to increased stress so that permanent lesions such as degenerative joint disease of the hip and knee may occur.

Because of the above, many authorities in sport have concluded that the potential dangers to the health of athletes by far outweigh the questionable advantages of anabolic steroid administration.

At a symposium held in London in 1975, representatives of the International Federation of Sports Medicine and of the medical commissions of international federations unanimously condemned the medical prescription of anabolic steroids to healthy athletes".

Stimulant drugs are taken just before competition to enhance performance. On the other hand anabolic steroids are taken over long periods and during training. Thus the dope control for stimulant drugs at a competition is effective to detect misuse. However, those misusing anabolic steroids could discontinue their use a

week or two before a particular event and then, when tested at the event, would be negative. The competitor would still have the weight gain and therefore some people have said that anabolic steroid tests are a waste of time. However, if a competitor discontinues the misuse of anabolic steroids then his performance drops dramatically because the effect of anabolic steroids are not only for weight gain but their central nervous system effect produces increased competitiveness. Discontinuing their use leads to loss of this competitiveness and even depression and thus lower performance. Some people and some countries are at present overcoming this disadvantage of having to stop before an event by injecting the male hormone testosterone; although this drug can be detected, the fact that this is also an endogenous material means at present we cannot act. However, there is a possibility that since the injection of testosterone influences other circulating substances that an indirect method of detection of misuse of testosterone will be found in the near future.

THE EFFECTS OF DOPE CONTROL

Since the introduction of dope control for stimulants and narcotics there has been a dramatic decrease in their misuse. This has occurred in those sports in which the federations have taken a clear action and instituted testing at various national and international events.

At the same time that this type of drug misuse was decreasing the misuse of anabolic steroids was escalating because of the absence of a test and thus the inability to take action. The introduction now of tests will certainly lead to a decrease in the use of anabolic steroids as long as the testing is carried out at main events and some system found for introducing testing in other events so that the misuse in training can be stopped.

In those sports in which there is no testing the misuse of stimulant drugs is still very great, for example in American football, where medical personnel in the teams have been involved. This continued and gross misuse is now coming into the open because of court cases.

One State Attorney has charged Dr. Mandell with unprofessional conduct, gross incompetence and gross negligence in prescribing amphetamines to 11 football players while he was team psychiatrist for the San Diego Chargers. Some of the points made by Dr. Mandell are as follows:

1. "Amphetamine abuse is the vocational disease of professional football."
2. "Author prescribed amphetamines to football players whom he knew were abusing the drug."
3. "Football players avoid the drug except before a game, when they ingest large amounts to induce a paranoid, psychotic and murderous rage. The drug eliminates the experience of pain, allowing them to play despite bruises, cracked ribs and swollen knees."

4. "The only way to wipe out amphetamine abuse in football is to require urine tests for all players before each game."

"This hasn't been done because it would eliminate most established football stars from the sport and would induce the violence that helps make football so popular. It's amazing the way Americans buy good tickets to watch speed freaks try to kill each other."

5. "From 1971, team trainers routinely handed out envelopes of benzedrine and dexedrine before a game. Since then football players have turned to team physicians and the black market to obtain amphetamines."

APPENDIX

ANALYSIS OF SAMPLES

The modern screening procedures for the detection of drugs of abuse rely heavily on various chromatographic techniques. An aliquot, usually 5 ml of the urine sample is first saturated with sodium chloride and then extracted into a suitable organic solvent, usually ether, under alkaline conditions to separate any basic drugs from normal urinary components. The addition of sodium chloride aids the extraction. The ether extract may then be concentrated by evaporation and subjected to gas liquid chromatography (GLC). As a quality control check on the system, an internal standard should be added preferably to the urine sample. Diphenylamine, which will not be confused with a drug of abuse, is often used for this purpose.

The initial screen makes use of SE30 or OV1 as a non-polar liquid phase or Apiezon-L with KOH and the temperature of the chromatograph oven is programmed to run from about 130°C to 280°C at a rate of over 15°C per minute. Using a nitrogen sensitive detector (NPD) single column operation may be employed since column bleed will not produce a signal with this detector which is selective towards nitrogen containing compounds. Alternatively, if the older type of flame ionisation detector (FID) is used then a second identical column must be connected to a dual detector to compensate for the varying column bleed which occurs during the temperature programmed run. The NPD has the additional advantage that components eluting close to the solvent are not masked by the solvent tail since the non-nitrogen containing solvents produce very little detector signal. The modern NPD is now sufficiently reliable and stable to allow unattended operation of the equipment. This screen is designed to be rapid and a total cycle time of less than 20 minutes may be obtained, allowing more than 3 analyses per hour per chromatograph. A suspect positive is indicated if a peak is observed on the chromatograph's chart recorder, or on the connected data system's output, during the analytical cycle which is not attributable to normal artifacts such as nicotine from smoker's urine or caffeine from coffee.

Should a suspect positive be indicated then confirmation is achieved by determination of Kováts indices using at least one other column, the preparation of a suitable derivative such as a Schiff's base with acetone or a trifluoroacetyl (TFA) derivative with trifluoroacetic anhydride (and see alternative GLC procedure) and finally using gas chromatography coupled mass spectrometry (GC MS).

THIN-LAYER CHROMATOGRAPHIC (TLC) SCREENING FOR STRYCHNINE

A further ether extract obtained as described above is concentrated and quantitatively transferred onto a glass plate pre-coated with a thin layer of Silica Gel G F₂₅₄. The plate is developed by dipping the bottom edge into a suitable solvent system such as ethylacetate: methanol: concentrated ammonia (70:25:5 by volume) in a closed TLC tank. The developed plates are air dried and then examined under ultra-violet light of 254 nm wavelength. The coating of the plate fluoresces and dark spots, caused by fluorescence quenching, are observed if certain compounds are present. The plates may then be visualised by spraying, with iodoplatinate reagent when any strychnine present appears as a distinctive deep violet spot at a position on the plate (the R_f value) appropriate to the compound as determined by the use of a pure reference solution of strychnine analysed in a similar manner.

GLC SCREEN FOR NON-VOLATILE DRUGS OF ABUSE

Some drugs of abuse, such as the narcotic analgesics, and many of their metabolic products are polyfunctional and relatively polar molecules which require derivatisation if they are to be analysed by GLC. Trifluoroacetylation is a useful derivatisation method for these compounds. The urine is extracted with diethylether to which 10% v/v propan-2-ol has been added to enable the more polar compounds to be extracted. The organic phase is then dried and derivatised with trifluoroacetic anhydride in ethylacetate. The excess reagent is removed by drying either under vacuum or in a dry nitrogen stream and the sample re-dissolved in anhydrous ethyl acetate. The derivatised sample may then be conveniently analysed by GLC using OV17 or similar semi-polar liquid phase in a temperature programmed run similar to the normal GLC screen. An internal standard such as phenazine is frequently used. The use of this second GLC technique has the added advantage of helping to confirm any suspect positives indicated by the first method described.

DETECTION OF DRUG CONJUGATES

Glucuronides or ethereal sulphates are hydrolysed to liberate the free drug by heating the urine sample to 100°C with concentrated hydrochloric acid. The solution is neutralised with alkali and then extracted with ether/propan-2-ol (9 : 1) as described above.

RADIO IMMUNOASSAY SCREEN FOR EXOGENOUS ANABOLIC STEROIDS

The immunoassay technique relying as it does on the complexing of anti-bodies with specific functional groups of the steroid molecule enables a sensitive assay to be developed. The method of choice for the routine screening for anabolic steroids is still essentially the same as that described by Brooks and co-workers in 1975. Two different antisera are usually used, one for the 17 α -alkyl substituted steroids which are usually taken orally and the other sensitive to 19-nortestosterone and related steroids which are usually administered by injection. Standard curves and the cross reactivities are usually determined using urine samples containing known quantities of the steroids of interest. A blank value is chosen after analysing a number of drug-free urine samples from normal volunteers. The assay is

Fig. 6: Characteristic mass spectrum of morphine showing the most intense ion peak of mass 285 corresponding to the molecular weight and other intense ion peaks corresponding to the compound's structural units.

rather time consuming, taking at least two days, and false positives may occur. However, the method is still comparatively cheap and rapid compared with the necessary mass spectrometric confirmatory test. Thus the immunoassay screen is useful to minimise the number of samples which must be subjected to mass spectrometry for the confirmation or rejection of the suspected presence of an exogenous anabolic steroid.

CONFIRMATORY TEST USING GAS CHROMATOGRAPHY COUPLED MASS SPECTROMETRY

The ionisation and subsequent fragmentation of sample molecules introduced into the mass spectrometer produces a spectrum, such as that shown in figure 6, which is so characteristic of the compound concerned as to enable a suspect positive to be confirmed unequivocally.

With the possible exception of the anabolic steroids the concentration of drugs of abuse in the urine sample is normally sufficiently large to enable a complete mass spectrum to be obtained. No general method for all compounds exists at the present time but initially an electron impact (EI) induced mass spectrum (MS) is obtained. In addition, it is often useful to obtain the EI — MS of the TFA derivative, if the compound will derivatise, since this derivative often gives rise to characteristic ions. In addition, a chemical ionisation MS, especially using isobutane as the reagent gas, may be of value. It is essential that the spectra be evaluated by a scientist experienced in mass spectrometry to ensure that the technique is used correctly to obtain an unequivocal confirmation of the suspect positive.

CONFIRMATORY TEST FOR THE PRESENCE OF EXOGENOUS ANABOLIC STEROIDS USING GAS CHROMATOGRAPHY COUPLED MASS SPECTROMETRY

This test was originally described by Ward and co — workers

in 1975 and requires the hydrolysis of any steroid glucuronide present in the urine sample with β — glucuronidase. This hydrolysis step is followed by initial concentration and purification by column chromatography using XAD-2 resin followed by further purification using a Sephadex LH — 20 column. The purified extract is then derivatised by trimethylsilylation and subjected to GC — MS. Dugal and Bertrand (1978) describe a rather more rapid method using a 9 : 1 v/v mixture of dichloromethane and isopropanol to extract any unconjugated steroids from the urine. The organic phase is evaporated to dryness, any steroid present is trimethylsilylated and subjected to GC — MS to confirm the presence of any 17 α -methyl substituted steroids. The aqueous phase remaining after the initial extraction process is subjected to enzymatic hydrolysis then re-extracted with a mixture of chloroform and ethyl acetate (4 : 1). The organic phase is evaporated and any steroids present are trimethylsilylated and subjected to GC — MS to confirm the presence of metabolites of 17 α -ethyl substituted steroids or of 19 nortestosterone. This simpler procedure has the disadvantage that many impurities are also extracted giving rise to larger backgrounds in the mass spectra obtained. However, this problem is largely overcome by recording not a complete mass spectrum of each compound eluting from the GC into the MS but instead by monitoring pre-selected ions which are known to be present in the spectrum of the compounds of interest. This technique has the added advantage of increased sensitivity since time is not being wasted monitoring irrelevant regions of the mass spectrum. However, it is essential that ions are monitored which are diagnostic of the exogenous anabolic steroids if a confirmation of their presence is to be obtained. Having selected the correct ions, and usually up to four ions may be monitored concurrently, then not only must these ions all increase in monitored intensity at the correct retention time for the suspected steroid but also the relative intensity of these ion peaks must be comparable with that obtained from an authentic sample. In addition, it is desirable to repeat the GC — MS analysis using the chemical ionisation mode when intense protonated molecular ions should be observed at

the correct retention time. The $(M + 29)^+$, $(M + 1 - 15)^+$ and $(M + 1 - 90)^+$ ions may also be usefully monitored at the same time.

TEST FOR ALCOHOL

Where a test for the presence of blood alcohol is required, a breath sample is tested immediately after the competition using a standard breathalyzer. If this screen indicates the presence of

more than 50 mg per cent of alcohol a blood sample is taken. A head-space analyser GC is used to determine the concentration of ethyl alcohol in the blood sample. In this technique the blood sample is transferred quantitatively into a suitable sample bottle together with an internal standard, such as tertiary butanol. This bottle is sealed and equilibrated to 60°C when a needle, connected to the gas chromatograph column, is made to pierce the bottle cap allowing the head-space vapours to pass into the chromatograph.

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