Testing for the misuse of anabolic steroids took place at the Olympic Games held in Montreal, 1976. In agreement with the IOC medical Commission and eight International Sports Federations some samples of urine were collected before the opening of the Games from competitors selected randomly from these particular sports; three disqualifications resulted.

During the Games, the IOC Executive approved the proposal of the IOC Medical Commission that samples taken during the Games under the normal procedures of selection for normal dope control should be subjected to anabolic steroid testing at the completion of the Games; these tests led to five further disqualifications including medal winners.

The control procedures for sampling and samples, and those for the re-check involving the B samples of urine, are subject to exactly the same inspection and verification procedures as adopted for “normal” dope control.

Undoubtedly, problems exist at present because of the long time needed between collection of urine samples and the completion of the first analysis and re-check analysis for the B sample when large numbers of samples are being processed for anabolic steroid control. Current research will shorten this time and also will increase the duration of the period between discontinuing the use of anabolic steroid misuse and the ability to detect the drugs in urine of a competitor chosen for dope control.

A. B.

Introduction

The rapid weight increase occurring at puberty is caused by increased secretion of sex steroid hormones, testosterone in males and oestrogens and progesterones in females. Testosterone production leads to the more muscular male, i.e. it enables nitrogenous compounds to be utilised to make body protein including muscle (its anabolic action) as well as behaving as a male hormone in exhibiting virilising actions (its androgenic action). Its administration to women causes the development of masculine characteristics, i.e. increase of muscle,
growth of hair, deepening of the voice, increase in greasiness of the skin.

Testosterone is a steroid (see Fig. 1); chemical modifications of the nucleus can partially separate the anabolic from the virilising actions of the compound. The natural hormone is metabolised to inactive compounds when given by the oral route and must therefore be given by injection. The introduction of a 17-α-alkyl substituent produces compounds e.g. methyltestosterone (Fig. 3) which are not metabolised as quickly by the oral route and these compounds are then active when given orally (see Fig. 3 for some examples of anabolically orally active steroids). This change also causes some separation of anabolic action from virilising action.
Testosterone

Fig. 1

2) but not orally unless the 17-$\alpha$-alkyl group is also introduced (see Fig. 3 for some examples).

**Use in athletics**

In the 1930s the anabolic action of testosterone in laboratory animals was reported. Subsequently the above synthetic compounds were prepared in an attempt to provide drugs in which the anabolic action was enhanced and the virilising action decreased; thus “anabolic steroids” were introduced into medicine as drugs to retain organic nitrogen in the body to build up body protein in underweight patients.

Athletes, especially those in throwing events, began to use them in the late 1950s in an attempt to improve performance. By 1964 and at the Olympic Games in Tokyo, much information was circulating concerning the misuse of these drugs in sport. The former Olympic hammer champion said in 1973, to

Alteration of the A ring of methyltestosterone reduces greatly the virilising effect (see Fig. 3) and providing there is a 17-$\alpha$-alkyl group present, the compounds are active when given orally (see Fig. 3 for some examples).

The removal of the 19-methyl group of testosterone (see Fig. 1) to give the 19-norsteroids (see Fig. 2) maintains the anabolic actions but reduces the virilising ones. These compounds are active parenterally (Fig.

**Anabolic Steroids Active Orally**

- Methyltestosterone
- Methandienone (Dianabol)
- Stanazolol (Stromba)
- 17$\alpha$-ethyl-4-estren-17$\beta$-ol-3-one (Nilevar)
- 17$\alpha$-ethyl-4-estren-17$\beta$-ol (Orabolin)

**Anabolic Steroids Active**

- Methenolone oenanthate (Primobolan)
- Stanolone (Androlone, Neodrol)
- Testosterone
- 19-nortestosterone
- Nandrolone phenylpropionate $R = OC(CH_2)_2 Ph$ (Durabolin)
- Nandrolone decanoate $R = OC C_9 H_9$ (Decadurabolin)

Fig. 2

Fig. 3

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a sub-committee of a U.S. Senate Committee hearing, “just prior to the 1964 Olympic Games in Tokyo, all around me it seemed that more and more athletes were using steroids for athletic preparation and one began to feel that he was placing himself in a decided disadvantage if he did not also get on the sports medicine bandwagon”. At this stage it was primarily only the athletes from “pharmacologically sophisticated countries” who were involved.

Since 1964, the misuse of anabolic steroids has spread rapidly to many countries and almost all branches of sport. For instance it was recently reported (Ljungovist 1975) that in Sweden in 1973, 75 per cent of all throwers were using these drugs and 31 per cent of athletes as a whole of those who completed the questionnaire.

Do anabolic steroids improve performance?

There is conflicting information about the effectiveness of anabolic steroids to improve performance. When trained athletes were tested, the evidence of O’Shea (1971), of Johnson et al (1972) and of Freed and Banks (1975) indicates that there is an improvement of athletic performance when these drugs are used. This is in contrast to the result of Fowler et al (1965) and Casner et al (1971) who found no improvement from their studies using healthy young men who were not highly trained athletes. The use of anabolic steroids and a high protein diet by athletes leads to weight gain but much of this is water due to fluid retention. However, because in throwing events the mechanism is associated with mass and momentum this type of weight gain can be advantageous.

The use of anabolic steroids has been reported by athletes to reduce fatigue and to allow more frequent and more strenuous training sessions. The incidence of injuries in training is stated to be reduced and quicker recovery after injury is reported. However, scientific evaluation is lacking.

It is possible that the adrenogenic effects of these drugs on the central nervous system may confer some advantages to the users

ANABOLIC STEROIDS - METHOD OF TESTING

Conjugate preparation

1. Form steroid-3-(O-carboxymethyl) oxime derivative of the steroid.
2. Form mixed anhydride and then link with human serum albumin to form conjugate.

Immunisation

1. Conjugate made into an emulsion and injected into rabbits—subcutaneously into 10-15 dorsal sites.
2. Booster dose given after 6 weeks—subcutaneously into 3 dorsal sites.
3. Sample of blood from ear vein checked for titre and specificity.
4. Blood collected, allowed to clot, serum obtained, sodium azide added as preservative and the product i.e. antiserum stored at 4°C until required.

Preparation of the radioactive label

1. Form steroid-oxime mixed anhydride.
2. Iodinate histamine with labelled iodine.
3. Conjugate the iodinated histamine to the steroid-oxime mixed anhydride.
4. The ¹²⁵I-labelled histamine-steroid conjugate is purified by thin layer chromatography.
5. Product eluted with ethanol and stored as an ethanol solution.
6. For assay purposes, a portion of the solution is taken, evaporated and the product redissolved in the phosphate/albumin assay buffer.

Fig. 4
because of increased aggressiveness and competitiveness, and because of euphoric effects and reduced pain sensitivity.

**What are the side effects and dangers of using anabolic steroids?**

The side effects of anabolic steroids on sex organs in animals have been clearly established. In the rat, they cause reduction in spermatogenesis and have marked effects on the testes and accessory sex organs, Kincl *et al* (1965); more recently Rogozkin (1975) has also shown weight reduction of the prostate, testicles and seminal vesicles and decrease in secretion in the seminal vesicles in rats. The output of testosterone and gonadotrophins is reduced by large doses of anabolic steroids in rats (Boris *et al*, 1969). It is to be expected that anabolic steroids will affect the sex organs in man; in two patients taking large doses of the anabolic steroids, oxymethalone and methandrostenolone, Harkness *et al* (1975) were able to show a reduction in testosterone and gonadotrophin output and some indication of reduction in spermatogenesis.

Anabolic steroids can also cause effects on the liver. Orally active anabolic steroids show liver toxicity and can cause jaundice (Wynn, 1975). The long use of anabolic steroids may be associated with liver tumours including cancer (Farrell, 1975).

There have been reports of jaundice, prostatism, hypertension, gastro-intestinal haemorrhage, decrease but sometimes increase of libido and oligospermia associated with the use of anabolic steroids (Freed and Banks, 1975). In studies using thirteen highly-trained athletes receiving 10 mg or 25 mg of methandienone per day, Freed *et al* (1975) showed a high incidence of side effects including headache, hypertension, urinary trouble, and raised levels of serum cholesterol and alanine transaminase. Also cholesterol levels were elevated but this may be a result of the diet rather than the drug effect.

Wynn (1975) has reported that orally active anabolic steroids produce a “host of unwanted effects and should really be regarded as highly dangerous compounds which should not be used except under careful medical supervision and even then they have only a restricted place in therapy”.

**Radioimmunoassay of Anabolic Steroids**

Fig. 5

They cause disturbances in carbohydrate and lipid metabolism and such drugs can be expected to yield to an accelerated development of atherosclerosis which leads to heart disease, strokes and peripheral vascular diseases. For instance methandienone in ten healthy subjects gave a deterioration in glucose tolerance associated with a significant fall of the fasting blood sugar; circulating plasma levels of triglyceride were increased despite enhanced triglyceride removal in five female subjects. On the other hand, the injectable anabolic steroids produce few side effects unless large amounts are used which then produce virilisation in women so that menstrual trouble, hirsutism and deepening of the voice may occur.
The use of anabolic steroids in pre-pubertal children can lead to premature sealing of the epipheseal plates of long bones with resultant stopping of growth. Although muscle mass is increased by anabolic steroids misuse under conditions of high protein diet, increase in tendon strength does not occur at a similar rate; thus torn tendons may result during vigorous exercise.

Conclusions on effectiveness and dangers of use of anabolic steroids in sport

At a F.I.M.S. international sponsored symposium held in London in 1975 on “Anabolic Steroids in Sport”, the following conclusions were made:

1. The actions of Anabolic Steroids in healthy, training athletes are not fully understood. Studies show conflicting results in increase in body size, measures of strength and improvement in performance. The use of Anabolics appears, however, to be widespread in certain sports. The difficulties of trial procedures are noted, especially in respect of ethical considerations.

2. Biochemical studies demonstrate side effects of Anabolic Steroids such as gonadal and pituitary suppression, and hepatic and prostatic involvement. In addition, psychological effects should be considered. The muscle bulk increase due to Anabolics is due mainly to water retention.

3. Detection methods for Anabolic Steroids, as well as naturally occurring hormones, are effective and we seek the wider spread of approved testing laboratories to eliminate the use of drugs in sport.

This Symposium was followed by a meeting of the F.I.M.S Technical Commission, including representatives of AGFI/GAIF (General Assembly of International Federations) at which the following matters received approval:

1. This meeting, considering the evidence of the London symposium (12.2.75) and previous evidence, condemns the prescription of Anabolic Steroids by physicians for healthy persons participating in sport.

2. This meeting recommends the International Federations to implement effective Anabolic Steroid control methods relevant to each sport throughout the year.

Detection of anabolic steroids

It was decided to use methods involving urine as the biological fluid, as in the case of dope control for other drugs.

Many of the anabolic steroids are extensively metabolised in man and conjugates e.g. glucuronides or sulphates of the parent drug and its metabolites are excreted in the urine.
ANABOLIC STEROIDS - RADIOIMMUNOASSAY

1. Diluted antiserum (0.3 ml) is added to pre-cooled plastic tubes (antibodies coat on plastic tubes).
2. Contents of tube aspirated, and the tubes washed with phosphate-albumin buffer (0.3 ml).
4. Then add to these tubes 0.1 ml of buffer alone, or sample in assay buffer, or standard in assay buffer.
5. After 1 to 2 hours is added to each tube steroid-125 I-histamine in assay buffer (0.1 ml). Contents mixed and kept at 4°C overnight.
6. Contents of each tube aspirated and the tubes washed with tap water (0.3 ml).
7. The wash is aspirated and the tubes counted in a gamma ray spectrometer.

Fig. 7

rather than the free form of these compounds. However, a small percentage of unchanged drug is excreted in the urine in the case of nearly all anabolic steroids.

The anabolic steroids are structurally slightly different from the natural steroidal hormones; use is made of these differences in the screening tests and in the definitive identification.

Screening tests

Radioimmunoassay techniques are very sensitive; Brooks and co-workers (1975), Sumner (1974) developed antisera (see Fig. 4) with the desired specificity for certain characteristic features of various types of anabolic steroids.

The appropriate antibodies were made by attaching an anabolic steroid e.g. 17-α-methyltestosterone by covalent links to a pure protein and injecting the product into rabbits. The animals produced antibodies against this foreign material; the antiserum so produced reacts not only with 17-α-methyltestosterone but also with other 17-α-steroids e.g. methandienone, stanozolal and oxymethalone (see Fig. 5), but not with 17-α-ethyl anabolic steroids. There was some slight cross reaction with testosterone but not with testosterone acetate; by suitable derivitisation it was possible to make the test specific for 17-α-methyl steroids.

Similarly 17-α-ethyl steroidal antisera were made using norethandrolone-protein as the antigen (see Fig. 5).

Recently, antisera against the 19-nor steroids used by injection (see Fig. 2) have been developed (Brooks, private communication).

The screening method (see Fig. 6) involves the extraction of a small sample of urine with dichloromethane, washing the extract, evaporating and drying, acetylation of the products, dissolving the acetylated material in buffer solution and then subjecting the solution to the radioimmunoassay (see Fig. 7). In this latter step use is made of the fact that antibodies can be coated on to plastic tubes so that rapid separation of free and bound steroids is possible. The tubes are counted finally in a gamma ray spectrometer; counts of standard, sample and zero links are made and the calculations carried out according to Fig. 8.

This method is simple and very sensitive but requires three days to complete under normal working conditions. It is only regarded as a screening test because the actual anabolic steroid present is not identified but only the class of anabolic steroid.

Definitive tests

Definitive tests are based upon gas-chromatographic—mass spectrometric methods for the parent drug and for its metabolites in urine (see Ward et al, 1975).

In general, this test is only used when the radioimmunoassay has given positive results.
The steroids are extracted from urine using an ion-exchange resin, fractionated and converted into trimethylsilyl ethers before analysis. Mass spectra for methandienone ("Dianabol") and for its main metabolite in urine are shown in Fig. 9.

These determinations are time consuming and involve expansion equipment. However, the result is unequivocal.

The enforcement of the anti-doping rules on anabolic steroids

The problem of preventing anabolic steroid misuse is different from the one involving stimulant drugs. If the latter are taken just before a competition, they will give a positive result in a urine sample taken immediately after the sporting event. However, a competitor may take anabolic steroids during training, and then discontinue their use two to three weeks before a particular event; a urine sample collected at the event may not show a positive result even though the competitor may still be having an advantage at least in weight from the drug misuse.

Despite these problems and the problems of analysis, the IAAF included anabolic steroids in its list of banned classes of drugs in 1970. Because these rules require the actual doping substance to be identified, the gas chromatographic/mass spectrometric technique was required and unfortunately not all drugs had been examined by this technique to produce definitive tests. Thus action was not taken on results which for a few years represented research operations.

In 1974, the IOC Medical Commission banned the use of anabolic steroids and at first defined a positive result as "failure to pass a test for the absence of anabolic steroids in urine as measured by a radioimmune assay based upon the use of a number of antisera and selectivity for structures in anabolic steroid molecules". At this time the gas-liquid chromatographic/mass spectrometric methods had not been developed sufficiently.

In April 1975, the IOC Medical Commission designated the radioimmune assay as a screening test only, and gas-liquid chromatography/mass spectrometry evidence to establish unequivocally the structure of the steroid used was made mandatory. It was agreed that testing for anabolic steroids must be carried out for the 1976 Olympic Games in Montreal.

The IAAF has encouraged its constituent bodies to hold anti-doping tests for anabolic steroids at all major international meetings. Testing has occurred at European Cup competitions and positive results have been obtained.

It is recognised that athletes could still misuse anabolic steroids with impunity if they discontinued this use two to three weeks before their major international meetings. However, this would certainly interfere with their training performances and discontinuing their use temporarily would produce some central nervous system depression which would tend to cancel out any potential benefit from their previous misuse.

Fig. 8

Anabolic steroids - Radioimmuno assay calculation

1. Number of counts in tubes of
   (a) buffer tube (antibody + labelled steroid) \( C_0 \)
   (b) sample tube (sample + antibody + steroid) \( C_s \)
   (c) standard tube (standard + antibody + labelled steroid) \( C_{ST} \)

2. Counts related on a percentage basis.

3. \( C_{ST} / C_0 \) plotted against amount of standard on log-linear graph paper to produce a STANDARD CURVE.

4. AMOUNTS IN SAMPLES then read from the STANDARD CURVE using the \( C_s / C_0 \) obtained for each sample.
Why should anabolic steroids be banned?

There are a number of ethical reasons:

1. The use of drugs contravenes the basic characteristics of sport which should involve the matching of skill and strength based upon the natural capabilities of the participant.

2. Competition in sport should involve the matching of skills of competitors and not of pharmacologists and physicians using competitors as guinea-pigs.

3. It is medically wrong to give a drug to a healthy person except for scientific purposes and when informed consent has been obtained.

In addition there are equally important practical grounds:

1. Anabolic steroids have many side effects even when single doses are given. The long term effects of massive doses taken for long periods may have serious consequences which may be revealed many years after the misuse has ceased.

2. The reports of increased aggression caused by anabolic steroids may place other competitors at risk.

It has been recorded by Payne (1975): “There are, undoubtedly, psychological effects resulting from steroid taking, and the ones who benefit most from these effects are the nervous, poor competitors, who suddenly find the confidence to lift themselves out of their mediocrity. They are the ones who would not like to have to come off the drugs if effective tests are brought in. The majority of athletes I have spoken to, however, would welcome the tests if they could ensure that no drug taker in the world can escape detection. The athletes themselves on the whole want to come off the steroids”.

The administrators in sport and the scientists and physicians in dope control bear a heavy responsibility to ensure that sport is cleansed from the cancerous growth of misuse of anabolic steroids in sport.

A.H.B.