Tobin

ANABOLIC STEROIDS: USE AND EXCRETION DATA

T. TOBIN, S. D. STANLEY AND J. P. GOODMAN

Acknowledgements: The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published as Kentucky Agricultural Experiment Station Article No. 89-4-28 with approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #169 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky.

Supported by grants from the Kentucky Equine Drug Research Council and the Kentucky State Racing and Harness Racing Commissions.

SUMMARY

The anabolic steroids are chemically related to testosterone, but are modified by synthetic chemists to enhance their anabolic or "tissue-building" actions while at the same time reducing their androgenic actions. Because of their origin in the testosterone molecule, all of these agents have some residual testosterone-like actions and none is exclusively anabolic in its pharmacology.

The anabolic group of drugs all have as their primary actions, in either man or the horse, the ability to increase anabolism, or synthesis of tissue. They therefore act to increase bone density and muscle mass, and may also act to increase muscle strength. They increase the haematocrit, and are recommended for this purpose in human medicine. Additionally, they all produce some of the characteristic behavioural effects of testosterone, increasing aggressive behaviour, herding, and mounting. In contrast with the anabolic effects, these behavioural effects develop rapidly, and are likely more closely related to blood levels of these agents than are the effects on bone and muscle mass.

The effects of these drugs on equine performance are not clearcut. The only rigorous study shows no performance effects of these drugs. This study, however, was performed with a small number of horses and would not be likely to detect small improvements in performance.

In contrast with the effects on performance, the effects on breeding stock are well characterized. In the male, the testicle is reduced in size and the sperm count may be reduced to the point that the animal is rendered infertile. In the female, the behavioural signs of oestrus are suppressed, and ovulation may be inhibited. Additionally, the behaviour of the animal becomes more male-like and clitoral enlargement occurs. These effects may take months to years to decay, depending on the type and amount of anabolic steroid administered.

The period for which anabolic steroids remain detectable in blood or urine similarly depends on the chemical nature of the drug administered and the nature of the testing methodology used. Recently very sensitive immunoassays for these agents have become available, and the principal determinant of the detection time for these agents is the availability of sensitive mass spectrometry confirmation methods.

INTRODUCTION

The anabolic steroids are drugs chemically derived from testosterone, the male sex hormone, in attempts to reduce the androgenic and accentuate the anabolic effects of testosterone (Wynn 1975). As shown by the changes in the pubertal male, testosterone has the ability to increase bone mass, muscle development and haematocrit in the male. Additionally, testosterone produces the changes in voice, body hair, and behaviour (mainly aggressive) that characterizes the adult male. This first group of effects, the effects on muscle mass, are called the anabolic or "body building" effects. The second group, changes in voice, hair and behaviour are called the androgenic actions.

Because of the likelihood of a strong market for a drug that could increase bone and muscle mass, many drug companies have taken the basic testosterone molecule and modified it in attempts to produce a drug with purely anabolic actions and no androgenic actions. These efforts have met with varying success, and it would be fair to say that none of these efforts has succeeded completely. Therefore, while all the anabolic drugs on the market have the ability to increase bone, muscle and body mass at the proper doses, they all have residual androgenic actions which modify the behaviour and actions of horses and which appear as side effects with respect to the desired androgenic actions of these drugs.

CHEMISTRY

The androgens are 19-carbon steroids of the testosterone family, with methyl groups bonded to carbons 10 and 13 of a four-ring system. The anabolic steroids include 19-nor compounds, which lack the methyl group at the A-B ring junction (Figure 1). There are two basic chemical types of synthetic steroids based upon the androstane and estrane ring systems. Those with a 17α-alkyl group are active orally, while those which have esterification of the 17-hydroxyl group (Haupt and Rovere 1984) are active parenterally and are the agents of choice among horse trainers and veterinarians. The presence of the ester group has a profound effect on the drug's pharmacokinetics (Figure 2). The acetates and propionates are rapidly absorbed and have a rapid effect with short term duration usually measurable in days. Longer in duration is the phenylpropionate, cyclopentylpropionate and undecylenate group. The effects of this group may last for weeks due to a slower rate of absorption. Boldenone (Equipoise®, Squibb Company, Princeton, NJ) is a member of this group. Finally, the laurates, decanoates, and heptanoates are very slowly absorbed and have the longest period of action, lasting for up to several weeks. Nandrolone decanoate (Deca-Durabolin®, Organon Company, West Orange, NJ) is in this category (Davis 1981). One other commonly used anabolic steroid which is not an ester is stanozolol (Winstrol-V®, Winthrop Labs, New York, NY). The anabolic steroids used in horses are generally oil-based suspensions that are administered intra-muscularly (Tobin 1981). Once these forms enter the horse's blood stream, they are rapidly hydrolyzed to their active ingredients (Davis 1981).

PHYSIOLOGICAL EFFECTS

The primary action of the anabolic steroids is to increase bone and muscle mass. One of the best characterized actions of this group of drugs is to increase the haematocrit, which action is most clearcut in the female and gelded male and is much less apparent in the entire horse. Similarly the anabolic steroids are thought to increase bone and muscle mass

in animals with a low circulating level of testosterone, and again the effect is most marked in the gelding and the female horse.

The actions of the androgen anabolic group of drugs on bone metabolism are very clearcut. They act to increase the retention of calcium in bones, giving rise to thicker bone and greater bone growth. However, the increase in length of the bone can only occur if the epiphyseal plates are still open and able to grow. Unfortunately, one of the actions of the anabolic steroids is to hasten the closure of epiphyseal plates and thus to prevent the growth of these bones. In the human, the castrated male or eunuch always grows taller and slimmer than the average male, precisely because of the lack of effect of endogenous testosterone on epiphyseal closure. Use of the anabolic-steroid androgen group of drugs in young horses before closure of the epiphyseal plates carries with it the danger of accelerating closure of these plates and preventing further growth.

As well as stimulating bone growth, this group of drugs also gives rise to increased muscle mass, and the significance of their actions on muscle mass and athletic performance

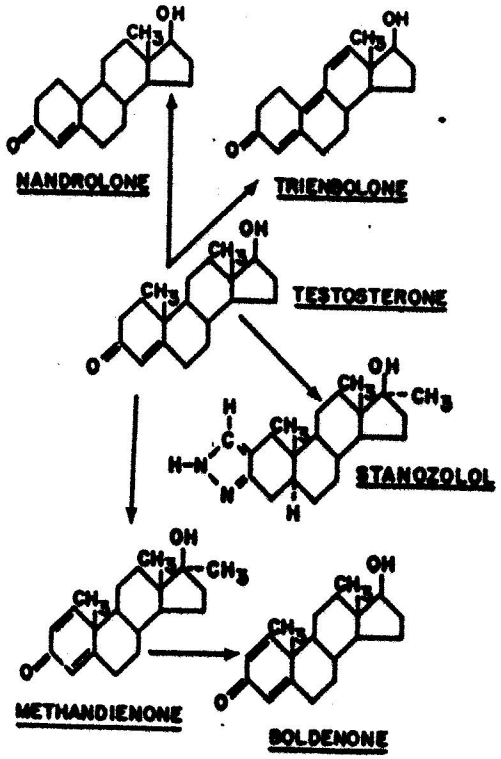

Figure 1:

Structure of testosterone and some anabolic steroids commonly used in equine medicine. Reproduced with permission from Charles C. Thomas, Publisher.

is a hotly argued area. These drugs may be used simply as "muscle puffers" to increase body mass and muscle, and also in the preparation of yearlings for sales. These uses are somewhat similar to the use of these agents by human body builders, amongst whom it is estimated that about 99 to 100% of contestants use these drugs. While it is not clear that use of these agents will actually increase muscle strength, it appears clear that they will increase muscle mass and add to the appearance of muscularity. However, convincing scientific evidence has not yet been developed to demonstrate unequivocal increases in muscular strength after administration of anabolic steroids. Review of the literature shows that some studies in this area have been interpreted as showing an increase in muscular strength, while others have been interpreted in terms of no increase in strength, but rather increases in muscle volume due to increased fluid retention, and with no parallel increase in strength.

Very closely interwoven with the effects of the anabolic steroids on body mass and performance are their psychological effects. Thus the androgen anabolic steroid group of drugs acts to produce marked feelings of well being and confidence, increase appetite and are important in the development of characteristic male aggressive behaviour. These effects are clearly seen in horses, which become more aggressive and difficult to handle when these drugs are administered to them. How much these effects influence athletic

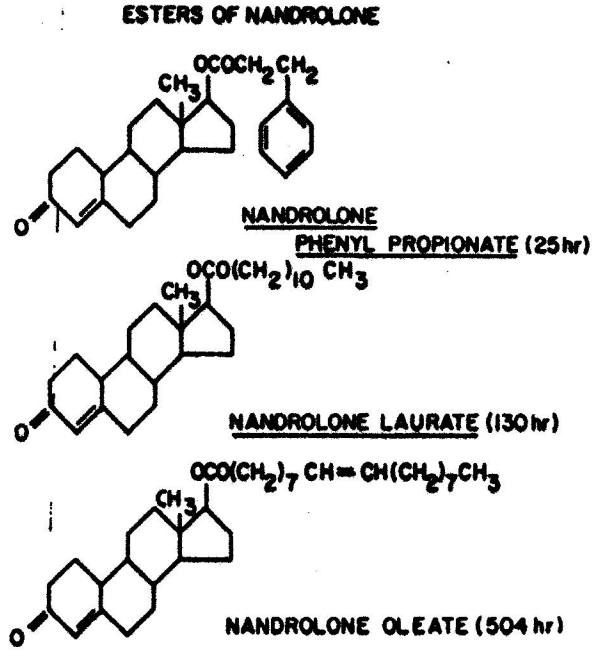

Figure 2:

The effect of different esters on the duration of blood levels of nandrolone, the figures in parentheses indicating the half-life of the compounds in rat plasma. Reproduced with permission from Charles C. Thomas, Publisher.

performance is not clear, but a strong feeling exists among human athletes that these drugs help a performer, giving a "winner's confidence" along with the will to win. There is no reason not to suppose that these effects also occur in animals and the consensus among trainers is that these drugs improve general vigor, muscle tone and hair coat, along with a more aggressive attitude.

THERAPEUTIC USES OF ANABOLIC STEROIDS

Possibly the primary use of anabolic steroids in the equine athlete is as an aid for treating debilitated horses when an improvement in weight, haircoat, or general condition is desired. This adverse condition often occurs following disease or may be due to overwork or overexertion. Positive results are produced since anabolic steroids create a more rapid positive nitrogen balance and secure a more efficient feed conversion.

Unfortunately, weight gains with use of anabolic steroids appear to be due mostly to retention of intracellular water and electrolytes. In such cases, effects are often short term; however, they may prove quite valuable when produced at the appropriate time. Another demonstrated action of the androgens is the increase of an animal's production of red blood cells and thus its haematocrit. In most species the entire male usually has a higher haematocrit than the female, while the haematocrit of the castrated male is lowest of all. Testosterone injected in the castrated male can produce up to a 20% increase in the red blood cell count. This effect also occurs in the female, and to a lesser extent in the entire male which usually has considerable amounts of testosterone already in circulation. Nevertheless, the effects of the androgen anabolic steroids on erythropoiesis is sufficiently clear-cut to render them the drug of choice in the treatment of anaemia, and in human medicine they are considered the most useful nonspecific stimulants of red blood cell formation (Tobin 1981). Other therapeutic uses are for correction of disorders such as parasitism, glucocorticoid-produced osteoporosis, fractures and post-operative surgery. In surgery, an important use of the anabolic steroids is in treatment of delayed healing, where they promote calcium deposition and aid in the repair of injured tendons. They may also be used after surgery simply to assist the animal in recovering from the stress of surgery (Tobin 1981).

NON-THERAPEUTIC USES OF ANABOLIC STEROIDS

The belief that anabolic steroids will increase athletic performance has led to their employment in the horse racing industry. Currently, there is conflicting evidence relating to effects of anabolic steroids on structural composition and athletic performance. Many physicians, veterinarians, and trainers attest to a gain in physical strength, mental attitude, and stamina following regular use of steroids. Some suggest that only one dose can show immediate effects on attitude and appetite. A strong feeling exists among athletes and trainers that anabolic steroids are a great help to the mediocre performer, giving him "winner's" confidence along with the will to win (Tobin 1981).

The advantages of increases in lean body mass, body weight, and performance have been difficult to document. Those studies that are available suggest that while some mild weight gain and lean body mass increases may be noted, athletic performance itself is not significantly altered by steroid use (Limbird 1985).

Although these studies indicate that anabolic steroids do not improve performance in all cases, it is possible that in individual animals an improved performance may result from their use. Their effectiveness in this respect may be due more to their androgenic effects on the central nervous system than to effects on the musculoskeletal system. It has been shown

that anabolic steroids retard the onset of fatigue in some cases; this may lead to an enhancement of performance through the ability of those horses to accommodate increased levels of training (Davis 1981).

Possibly the greatest effects of anabolic steroids occurs during the growth stages. Their favourable effects on young animals has created a use for these hormones on immature horses with the objective of improving muscular development to enhance their appearance and performance at the time of sale. Anabolic steroids used at this time may have the most dangerous side effect related to the use of these drugs. Examples of these side effects are restricted growth and reproductive failure (Snow *et al* 1977).

SIDE EFFECTS OF ANABOLIC STEROIDS

Normal developmental changes due to testosterone usually occur around the time of puberty. These changes are due to a natural increase in the production of testosterone in the still immature male. However, these same changes can be brought on prematurely by the use of anabolic steroids administered prior to normal maturity. Several undesirable side effects may then occur, the most severe being premature closure of the epiphyseal plate due to calcium deposition. Since bone mass and growth require weeks of therapy for full effects to develop, prolonged therapy may actually stunt or limit normal growth potential (Tobin 1978).

The action and effects of anabolic steroids on reproductive function has been studied in great detail. Work done at Colorado State University has shown conclusive evidence that anabolic steroids impair the ability of stallions and mares to successfully carry out proper reproductive function through the following breeding seasons. In young mares administration of steroids caused abnormal sexual behaviour. This was characterized by mounting other mares and aggressive behaviour toward other horses. They demonstrated that such behaviour was not seen in control mares which did not receive anabolic steroid (Maher *et al* 1983). Other stallion-like behavior such as teasing was frequently observed. These behavioural patterns persisted for several months after the steroid administration was discontinued. Other changes included enlargement of the clitoris, decrease in ovarian size, decrease in the number of large follicles, reduction in number of ovulations, and fewer oestrous cycles/mare than in the control group (Maher *et al* 1983). Overall, these effects rendered the treated mares much more difficult to get in foal. This was not due to a reduction in the mare's ability to become pregnant, but to the fact that oestrous had to be determined by rectal palpation, and artificial insemination had to be used since only about one-half of the treated mares would accept the stallions.

The administration of anabolic steroids to young stallions proved highly detrimental to semen quality, spermatozoal output and production, testis size, and concentration of luteinizing hormone (LH) in serum (Squires *et al* 1982). Administration of anabolic steroids reduced spermatozoal motility, concentration, and total sperm/ejaculate when compared with the non-dosed control group. The percentage of morphologically normal spermatozoa in ejaculates obtained was also lower. Most importantly was the substantial decrease in testes size which was observed in most of the anabolic steroid-treated stallions. These measurements proved inadequate to pass a breeding soundness examination (Figure 3). Two thirds of the test groups had depressed levels of circulatory LH, namely those receiving 1.1 mg nandrolone decanoate/kg and those receiving 4.4 mg boldenone undecylenate/kg. One group, that which received 1.1 mg boldenone undecylenate/kg showed increases initially, but later their level declined below those of the non-dosed

control group. Treatment with anabolic steroids had no effect on erection time, time to first mount, ejaculation time, or number of mounts/ejaculation. They additionally reported no beneficial effects of anabolic steroid treatment on libido. Because of the detrimental effects of anabolic steroid on reproductive function, their use is not recommended in stallions intended for breeding purposes (Squires *et al* 1982).

EXCRETION DATA

When looking for evidence of anabolic steroid administration, it must be recalled that there are two chemical classes based upon the androstane and oestrane ring systems, the 17 *a*-alkyl group and the 17-hydroxyl group. Most of the anabolic steroids used in horses do not have an alkyl group in the 17 position. Those commonly used are nandrolone, boldenone, trenbolone, and testosterone itself.

Figure 3:

Testicle size in control and anabolic steroid treated stallions. The testicle to the left is from a horse treated with the 4.4 mg boldenone undecylenate dose, while the testicle to the right is from a control horse. In some treated horses, testicular weight was reduced to around 40 percent of control weights. Reproduced with permission from International Equine Medication Control Group, Publisher.

The urinary excretion products of these compounds are present as glucuronic acid or sulphate conjugates. The a_{17} -hydroxy epimers occur mainly as glucuronic acid conjugates, and the 17 ß compounds are found mainly as sulphate conjugates (Dumasia and Houghton 1981).

Testosterone is inactivated primarily in the liver. Metabolism to androstenedione involves oxidation of the 17-hydroxyl group. In the process, reduction of ring A of androstenedione leads to formation of androstanedione, which results in greatly reduced activity. The keto group in the 3 position is reduced to form androsterone. A second possible pathway occurs when the 5 ß position is reduced in androstenedione and can undergo 3-keto reduction to form etiocholanolone. Dihydrotestosterone itself is converted in the liver to androsterone, androstanedione, and androstanediol.

The esters of testosterone are hydrolyzed to free testosterone and follow similar paths of metabolism. However, analogues of testosterone, eg. the 17-alkyl family, may follow different paths of metabolic degradation. Unaltered compounds, metabolites and conjugates are excreted in the urine and faeces (Dumasia and Houghton 1984).

REFERENCES

Davis, L. E.: (1981) — Anabolic steroids in the horse. *J. Am. Vet. Med. Assoc.* 179, 278-280.

Dumasia, M. and Houghton, E.: (1981) — Studies related to the metabolism of anabolic steroids in the horse: the identification of some 16-oxygenated metabolites of testosterone and a study of the phase II metabolism. *Xenobiotica.* 11, 323-331.

Dumasia, M. and Houghton, E.: (1984) — Studies related to the metabolism of anabolic steroids in the horse: the phase I. and phase II. biotransformation of 19-nortestosterone in the equine castrate. *Xenobiotica.* 14, 647-655.

Haupt, H. and Rovere, G.: (1984) — Anabolic steroids: a review of the literature. *Am. J. Sports. Med.* 12, 469-484

Limbird, T.: (1985) — Anabolic steroids in the training and treatment of athletes. *Comp. Therap.* 11, 25-30.

Maher, J., Squires, E., Voss, J. and Shideler, R.: (1983) — Effects of anabolic steroids on reproductive function of young mares. *J. Am. Vet. Med. Assoc.* 183, 519-524.

Squires, E., Tobter, G., Berndtson, W. and Pickett, B.: (1982) — Effect of anabolic steroids on reproductive function of young stallions. *J. Anim. Sci.* 54, 576-582.

Snow, D., Munro, C. and Nimmo, M.: (1977) — Anabolic steroids in equine practice. *Proc. Am. Assoc. Equine Pract.* 23, 411-418.

Tobin, T.: (1978) — Pharmacology review: the anabolic steroid androgen group of drugs. *J. Equine Med. Surg.* 2, 163-165.

Tobin, T.: (1981) — Drugs and the Performance Horse. Charles C. Thomas, Springfield, IL, pp 149-158.

Wynn, V.: (1975) — Metabolic effects of anabolic steroids. *Br. J. Sports Med.* 9, 60-64.