THE ABSORPTION, DISTRIBUTION AND EXCRETION OF ANABOLIC AGENTS

R. J. Heitzman

ARC Institute for Research on Animal Diseases, Compton, Newbury, Berkshire, RG16 ONN, United Kingdom

Summary

The metabolic fate of the anabolic agents, diethylstilbestrol, hexestrol, trenbolone acetate, zeranol and the endogenous steroids are discussed under the headings absorption, distribution and excretion. There is an optimum concentration of anabolic agent in the systemic circulation that results in a maximum increase in growth rate of farm animals. This optimum blood concentration should be maintained over a long period. However, there is rapid metabolism and excretion of anabolic agents with short half-lives in blood, and metabolic clearance rate equals entry rate. The rate of absorption of the agent, which is determined by formulation and site of administration, is most important and is best achieved by the use of slow release implants. The pattern of exponential absorption from compressed pellets of single anabolic agents is not ideal, and a more constant payout of drug, in particular estradiol-17β, is best achieved in combined preparations of agents or from silicone rubber implants impregnated with the agent. The high metabolic clearance rate and rapid excretion of anabolic substances influences the distribution of residues. Outside the site of administration, less than 1% of the administered dose is present in the animal. The lowest concentrations of residues are found in muscle and fat, higher concentrations are present in liver and kidney and the highest concentrations are in the bile, urine and feces.

(Key Words: Anabolic Agents, Residues, Farm Animals, Implants, Formulation.)

Introduction

Many factors influence the fate of anabolic agents in farm animals including: (a) absorption as determined by the site of administration and formulation of the agent; (b) distribution within the various body tissues or the disposition of the agent; (c) metabolism of the agent and (d) excretion of the agent and its metabolism.

Although the exact target tissues and intracellular receptors for anabolic agents have not been clearly identified, the pituitary gland and the muscle cell are probably the most important, while the metabolism of the agent occurs in the blood, liver and kidney. Anabolic agents, through their action on the pituitary gland, may act indirectly at the muscle cell by changing the concentrations of other endogenous anabolic and catabolic hormones, e.g., growth hormone, insulin, prolactin, thyroxine, triiodothyronine and corticosteroids.

Anabolic agents pass freely into and out of cells. Within the cells of the target tissues they occupy specific receptors and their anabolic activity is probably proportional to the number of occupied binding sites. When all of the sites are occupied, the receptors are saturated and an increase in the concentration of agent has no further effect. Thus, there is an optimum or "threshold" cell concentration of the anabolic agents that results in maximum cell response and, ultimately, maximum increase in physiologic response. Although this optimum intracellular concentration is unknown, it is assumed to be proportional to the concentration of agent in the surrounding blood. Thus, if the active agent is maintained at a concentration in the circulation that maintains the threshold concentration in the target cells, the result is optimum growth performance in farm animals. Thus, the opti-
Drug delivery system is one that maintains the concentration of anabolic agent at the threshold concentration throughout the treatment period. When the threshold concentration is exceeded excessive amounts of the agent will be absorbed, and there may even be undesirably high residues in edible tissues. Such a situation has occurred in Europe, where veal calves were illegally given massive doses of diethylstilbestrol (DES) by im injection.

Absorption of Anabolic Agent

Preparations of anabolic agents can be classified according to the following scheme:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Route of administration</th>
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<tr>
<td>Feed additive</td>
<td>Oral</td>
</tr>
<tr>
<td>Suspension - oil,</td>
<td>Im injection or sc injection</td>
</tr>
<tr>
<td>microcrystalline</td>
<td>sc implant</td>
</tr>
<tr>
<td>Compressed pellets,</td>
<td></td>
</tr>
<tr>
<td>impregnated silicone rubber</td>
<td></td>
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</tbody>
</table>

Feed additives have the advantage that their regular intake maintains constant concentrations of agent in the circulation. However, the choice of agents is limited because most are metabolized either in the gut or rumen or during their initial absorption via the portal system in the liver. Only the synthetic agent, melengestrol acetate for ruminants in the United States, is still used as a feed additive.

Injectable suspensions of anabolic agents, either oil based or microcrystalline, are frequently used for therapeutic purposes, but they are less suitable for use as growth promoters because they are absorbed too rapidly from their injection site. For example, the half-life of a 300 mg im injection of trenbolone acetate is 15 d in cattle (figure 1). The rate of absorption of trenbolone acetate given by injection is exponential (figure 1), and thus it is not generally possible to maintain a threshold concentration for a long period. Initially plasma concentration greatly exceeds the threshold concentration, and then falls too rapidly to be effective. However, the im injection of lipophilic long chain esters of anabolic agents, such as esters of 19-nortestosterone, prolongs the absorption time (Johndorf and Moss, 1978) and these preparations have been used in horses.

Compressed pellets and im injections of single anabolic agents exhibit similar exponential absorption kinetics, although their rate of dissolution is much slower. For example, the effective half-life of trenbolone acetate is 15 d for the injected form in oil, but is 60 d for the implant (figure 1). Implants of trenbolone acetate, zeranol, DES and hexestrol are widely used, and their effect on growth promotion persists for 2 to 4 mo after administration (Heitzman, 1979).

When compressed pellets are used containing a mixture of estradiol and a second steroid (trenbolone acetate, testosterone or progesterone) the absorption of estradiol from these combined implants is delayed even further and absorption maintained at a near constant rate for several months (Harrison, 1981). Plasma concentrations of estradiol in steers implanted with single or combined preparations of 140 mg trenbolone acetate and 20 mg estradiol are shown in figure 2. The delayed absorption of estradiol in the combined preparation was attributed to a physical interaction between the two steroids, because the absorption of estradiol placed separately in the opposite ear to trenbolone acetate was not different from the absorption of a single implant of estradiol (Heitzman et al., 1981). The same effect was observed in sheep given preparations of estradiol with trenbolone, progesterone or testosterone (Harrison, 1981). In steers treated with combined implants the increased concentrations of estradiol in plasma for the
longer period resulted in greater improvements in live weight gain (figure 3), feed conversion efficiency and carcass weights (Heitzman et al., 1981).

The sustained slow release of anabolic agents has been further developed by the introduction of a new delivery system for estradiol. This system uses silicone rubber implants impregnated with estradiol. Absorption rate of estradiol is proportional to the surface area of the silastic implant (Wagner et al., 1979). It is claimed by the manufacturer that these implants can be used for 400 d in steers. The relationship between growth responses in steers and average daily amount of agent absorbed over a 200 d trial period is shown in figure 4. The data suggest that the average entry rate of estradiol into a steer for optimum performance is about 55 μg/d. The entry rate of estradiol slowly decreased throughout the treatment period; e.g., it was 45 μg/d on d 84 and 34 μg/d on d 196 after implantation (Wagner et al., 1979). Turner et al. (1981) reported that steers given a single implant delivering an average of 47 μg estradiol/d gained 8% more weight per day than untreated steers during a 499 d trial.

**Metabolism and Disposition of Anabolic Agents**

The metabolism of anabolic agents has been described by Rico (1983). Anabolic agents are generally metabolized and the...
metabolites are either excreted or taken up by the tissues in a nonbiologically active form. There is a long exposure of drug in animals implanted with anabolic agents that may last several months. It can be assumed that a steady state exists between entry rate and metabolic clearance rate, the latter being determined by the rate of metabolism and excretion. Because of the high capacity of the liver and kidney to metabolize or excrete the circulating anabolic agent we can assume that clearance rate equals entry rate. Eighty percent of a 2.8 g dose of trenbolone acetate was cleared into the bile within 24 h after iv administration (Pottier et al., 1981), suggesting that the capacity of the liver to metabolize anabolic agents under field conditions is never exceeded, because most implants contain less than 300 mg of an androgen or 60 mg of an estrogen and these are absorbed over 60 to 150 d.

The half-life of many anabolic agents is measured in minutes rather than hours (Pottier et al., 1975; Harrison, 1981). Consequently anabolic agents must enter the circulation continuously to maintain effective concentrations in the target tissues. Very little is known about the metabolism of the active drug in the target tissues. The anabolic agent enters the target cell and has to form a complex with its receptor before a cellular response is initiated. Whether the agent is metabolized before or after complex formation is not known, but metabolism within the cell will affect the active concentration of the agent in the cell.

The disposition of anabolic agents has been determined by administering radiolabeled agents and measuring their metabolic fate in tissues, fluids and excreta. These radiometric studies have been reported for DES administered orally and parenterally to cattle (Aschbacher et al., 1975; Rumsey et al., 1975a,b), trenbolone acetate in cows (Pottier et al., 1975), estradiol in pigs (Bottoms et al., 1977), estradiol and estradiol benzoate in cattle (Dunn et al., 1977) and progesterone in cattle (Estergreen et al., 1977). All of these studies showed high concentrations of residues at the site of administration and in the bile, feces and urine. Higher concentrations of residues were found in liver and kidney than in other tissues. Further analysis of the nature of the radiolabeled residues indicated that there was considerable metabolism of the anabolic agents either to conjugates or metabolites with altered functional groups or to unidentified metabolites that were irreversibly bound to tissues (Rico, 1983).

Rumsey et al. (1975a) compared the effects of orally administered DES with those of implanted DES and concluded that the pattern of distribution of DES among feces, urine, tissues and bile was similar, suggesting that the DES excretion mechanism functions independently of route of administration. Those authors also concluded that the need for a much larger oral dose than an implanted dose to elicit a similar growth response is probably the result of the efficiency with which the liver metabolizes DES entering through the portal system.

**Excretion**

Anabolic agents are excreted in feces, urine and milk. Anabolic agents are not allowed in milking animals except in cull cows destined for meat. The liver converts anabolic agents to less biologically active metabolites and conjugates that are usually more water soluble. Those that are secreted in the bile enter the feces and are excreted. Some of the conjugated fraction may be hydrolyzed to the free form of the agent by bacteria in the gastrointestinal tract with the free form (e.g., DES) than excreted or reabsorbed via the enterohepatic circulation. Some of the metabolites formed in the liver may enter the systemic circulation and enter other tissues as residues. The role of the liver is very important in the metabolic fate of anabolic agents, but no studies have been conducted using farm animals.

Anabolic agents are metabolized and excreted from the kidney into the urine. The distribution of excreted radioactivity between urine and feces measured in the radiometric studies was not the same for all anabolic agents or species. For example, the excretion of radioactivity after administration of an implant of 3H-estradiol combined with trenbolone acetate to the veal calf is shown in figure 5. The concentration of residues in feces was higher than in urine, showing that feces was the preferred route of excretion, and there also was little change in the ratio of residues in feces and urine throughout the treatment period (Riis and Suress, 1976). On the other hand, after oral feeding of 14C-estradiol to swine, 75% of the radioactivity
was excreted in the urine within 72 h and less than 10% was excreted in the feces (Bottoms et al., 1977). When radiolabeled DES was administered to steers and sheep approximately 60 to 70% and 84 to 95%, respectively, of the excreted label appeared in the feces (Aschbacher and Thacker, 1972). These observations indicate the most suitable fluid, tissue or excreta to be used for the measurement of residues of anabolic agents. For example, it is not possible to detect residues of hexestrol in muscle or fat of steers implanted with 36 or 60 mg hexestrol, because the concentrations are less than 50 ppt, which is below the sensitivity of the radioimmunoassay method (Harwood et al., 1981). However, as depicted in figure 6, the presence of hexestrol in feces of similarly implanted steers was easily detectable throughout the treatment period.

Conclusion

There is rapid metabolism and excretion of anabolic agents with short half-lives in blood, and metabolic clearance rate is equal to entry rate. Therefore, formulation and route of administration, which determine entry rate, are most important in the use of anabolic agents. There is an optimum concentration of anabolic agent in the systemic circulation that results in maximum increase in the growth rate of farm animals. The optimum blood concentration should be maintained throughout the treatment period and is best achieved by slow release implants.

The pattern of exponential absorption from compressed pellets or injections is not ideal and high concentrations and residues are found in the early part of the treatment period.

The high metabolic clearance rate and rapid excretion of anabolic agents influences the distribution of residues. The lowest concentrations are found in muscle and fat, somewhat higher concentrations are present in the liver and kidney and the highest concentrations are in the bile, urine and feces.

Literature Cited


