Anabolic agents—some thoughts on their mode of action

By P. J. BUTTERY, B. G. VERNON and J. T. PEARSON, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

One of the early attempts to chemically manipulate growth was by Stotsenburg (1913) who studied the effects of gonadal hormones on growth. While the use of endogenous hormones, e.g. 17 β-estradiol, testosterone and progesterone has met with success, considerable interest has been shown in several synthetic compounds. e.g. zeranol [6-(6, 10-dihydroxy-undecyl)-β-resorcylic acid-μ-lactone], trenbolone acetate (3-oxo-17-β-hydroxy-4,9,11-estratriene acetate) and diethylstilbestrol (Van der Wal, 1976). The mode of action of these agents, especially the synthetic compounds is not fully understood. However, judging by their over-all effects upon metabolism they may be divided into two classes, those which have oestrogenic properties, e.g. diethylstilbestrol and those which have androgenic properties, e.g. trenbolone acetate. Naturally occurring androgens appear to have a direct anabolic effect upon muscle metabolism following combination with specific cytoplasmic receptors (King & Mainwaring, 1974). Oestrogens on the other hand, while inducing the production of specific proteins in tissues like the uterus, the liver and the chick oviduct do not appear to induce muscle protein deposition directly but presumably act by altering the pattern of endogenous anabolic hormones. It may be of considerable significance that, at least in the ruminant, oestrogens induce an increase in the plasma concentration of growth hormone, insulin and thyroxin (Preston 1975).

Diethylstilbestrol has been shown to have effects on the endogenous hormone status (Preston, 1975; Trenkle, 1976). The plasma concentrations of insulin and growth hormone were shown to be increased. It is attractive to speculate that the initial effect of diethylstilbestrol, or indeed any oestrogenic growth promoter, is to increase the secretion of growth hormone following an enhanced pituitary activity, since similar responses to those reported in diethylstilbestrol implanted sheep can be reproduced by the application of purified growth hormone (Davis *et al.* 1970). The elevated plasma insulin concentration might be explained by the reported control of insulin secretion by growth hormone both in vivo (Dawson & Hales, 1969) and in vitro (Curry *et al.* 1974). Both growth hormone and insulin have been shown to increase tissue amino acid uptake (Riggs, 1970; Manchester, 1971). The effect of insulin and growth hormone on tissue fractional synthetic and fractional degradative rates are conducive for growth (Goldberg *et al.* 1974). Insulin has also been reported to increase the activity of the initiation process of protein synthesis (Rannels *et al.* 1976; Jefferson *et al.* 1977; McKee *et al.* 1978). The above data are consistent with the elevated growth rates of diethylstilbestrol treated animals.

Changes in plasma amino acid concentrations are also consistent with these speculations in that Oltjen *et al.* (1973) reported that diethylstilbestrol treatment of steers reduced the plasma concentration of both the essential and the non-essential amino acids, as well as urea. Diethylstilbestrol has also been shown to increase the energetic efficiency of protein deposition (Fowler *et al.* 1970).

Another frequently employed oestrogenic growth promoter is Zeranol. Although the mode of action of this growth promoter is less clear than that reported for diethylstilbestrol, there is evidence for an elevation of the plasma growth hormone and insulin concentrations in treated animals (Sharp & Dyer, 1970; Borger *et al.* 1973).

Androgenic growth promoters have been reported to have no significant effect on the endogenous hormonal status of treated animals (King & Mainwaring, 1974) with the possible exception of thyroxin (Heitzman, 1976); results which are in contrast to that reported for oestrogenic compounds.

In an attempt to elucidate the mode of action of an androgenic growth promoter, Vernon & Buttery (1976, 1978*a*,*b*) studied the effect of trenbolone acetate on protein turnover in the female rat. (Trenbolone acetate seems to require the presence of an oestrogen in order to promote a significant growth response (Heitzman, 1976)). Trenbolone acetate appeared to reduce the rate of muscle protein synthesis (assessed by the technique described by Garlick & Marshall, 1972) and the rate of muscle protein breakdown (assessed both by the $Na_2\ ^{14}CO_3$ technique (Millward, 1970) and by the excretion of N-^{t}-methyl histidine (Vernon & Buttery, 1976, 1978*a*)) (see Table 1). Liver fractional synthetic rates were not

Table 1. ***The effect in the female rat of trenbolone acetate (80 μg/100 g body-wt/d) on the rate of protein turnover***

(Results are mean values with their standard errors)

	Test				Control			
	Fractional synthetic rate/d		Fractional degradative rate/d		Fractional synthetic rate/d		Fractional degradative rate/d	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Tyrosine infusions								
Liver	0·55	0·05	—	—	0·47	0·04	—	—
Heart	0·13	0·01	—	—	0·17	0·02 *	—	—
Uterus	0·33	0·02	—	—	0·53	0·06 **	—	—
Hind limb muscle	0·086	0·005	—	—	0·110	0·005**	—	—
$Na\ ^{14}CO_3$								
Hind limb muscle	—	—	0·048	0·001	—	—	0·060	0·001***
N-^{t}-methyl-histidine excretion								
Myofibrillar protein	—	—	0·012	0·001	—	—	0·017	0·001***

Significant differences between test and control group $^{*}P<0·05$, $^{**}P<0·01$, $^{***}P<0·001$.
For experimental details, see Vernon & Buttery (1976, 1978*a*).

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significantly changed (Vernon & Buttery, 1978*b*). A reduction in both fractional synthetic and degradative rates of muscle is of course conducive to growth provided the rate of synthesis still exceeds the rate of degradation. This would appear to be the case in the treated rats.

This reduction in protein synthetic activity was rather surprising since Barragry (1974) suggested that anabolic agents increase the rate of protein synthesis and decrease the rate of catabolism. Attempts to investigate the mode of action further have not been particularly fruitful. No significant differences in plasma amino acids have been noted between treated and untreated animals (Vernon & Buttery, 1978*b*). However the muscle intracellular concentrations of three amino acids glycine, lysine and arginine were found to be significantly altered (see Table 2) (Vernon & Buttery, 1978*b*). In this context it is of interest that trenbolone acetate treated rats show a marked increase in urinary cadavarine excretion (J. T. Pearson, unpublished observation). Cadavarine is of course the decarboxylation product of lysine.

Table 2. *The effect in the female rat of trenbolone acetate treatment (80 μg/100 g body wt/d) on the intracellular concentration of some amino acids (μmol/g wet wt) in muscle*

(Results are mean values with their standard errors, for six rats)

	Glycine		Lysine		Arginine	
	Mean	SE	Mean	SE	Mean	SE
Treated	5·06	0·56	0·58	0·76	0·13	0·02
Control	3·75	0·13	1·34	0·22	0·24	0·05

Differences between means significant ($P<0·05$) in all cases.
Values refer to 14 d after start of treatment.
For details, see Vernon & Buttery (1978*b*).

Polyamines have been strongly implicated in growth processes. Herbst & Snell (1948) reported that certain bacterial mutants had an absolute requirement for polyamines. Cohen (1971) implicated polyamines in the regulation of RNA synthesis. During studies by Vernon & Buttery (1978*b*) it was noted that trenbolone acetate caused a marked increase in the RNA content of skeletal muscle, an observation which is not entirely consistent with the concept of the activity of RNA (Millward *et al.* 1975).

Normally an increased RNA content is associated with an increased protein synthetic activity but a decrease in the fractional synthetic rate of muscle protein was accompanied by an increase in RNA content in the treated female rats. It is however most relevant to quote Russel (1973), 'To my knowledge no growth process will occur without the prior stimulation of polyamine biosynthesis'.

Despite the above information the exact mode of action of androgenic growth promoters remains unclear but the following possible mechanism could be advanced.

A derivative of trenbolone acetate (17 α-methyl trienolone) has been shown to bind to skeletal muscle testosterone receptors (Tremblay *et al.* 1977). There is evidence that the combination of glucocorticoids with their cytoplasmic receptor can be inhibited by the presence of testosterone (Mayer *et al.* 1974; Mayer & Rosen, 1975). Perhaps a trenbolone acetate metabolite and testosterone have similar metabolic effects. Millward *et al.* (1976) following 2 d of glucocorticoid treatment of rats noted a reduction in fractional synthetic rate and an increase in the fractional catabolic rate of skeletal muscle protein. It may therefore be that if this catabolic effect was partially blocked by the inhibition of the formation of the cytoplasmic-glucocorticoid-receptor-complex by a trenbolone acetate metabolite then an increase in muscle growth might result. This hypothesis does not entirely account for the situation with trenbolone acetate since on treatment a reduction in fractional synthetic rate of muscle is also seen.

Thyroxin has been implicated in the anabolic effect of both oestrogenic and androgenic growth promoters (Preston, 1975; Heitzman, 1976). However, since thyroxin has so many different effects upon tissue metabolism (Bernal & Refetoff, 1977) it is difficult to come to any concrete conclusions as to the significance of these observations.

There is little evidence for an effect of trenbolone acetate upon plasma growth hormone and insulin concentrations in treated ruminants (Galbraith & Miller, 1977; Heitzman, 1976) which is in contrast to the situation reported for oestrogenic growth promoters. The possibility remains that a trenbolone acetate metabolite may directly effect the tissues and one possible method is discussed above (i.e. the interference with the formation of the glucocorticoid-cytoplasmic-receptor-complex).

It is readily conceded that the effect of trenbolone acetate on the protein metabolism of the rat may not be identical to that seen in farm animals, especially ruminants. Indeed the responses of the rat to diethylstilbestrol have been reported to be different from those of cattle and sheep (Moffett *et al.* 1975).

While the information available at present does not enable the exact mode of action of anabolic agents to be postulated the increasing use of such agents must encourage more work to be undertaken. It may follow that when the mode of action of such agents is understood that efforts to design other and perhaps more effective materials may be more successful.

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