Protein turnover in rats treated with Trienbolone acetate

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1. The anabolic agent Trienbolone acetate (3-oxo-17-β-hydroxy-4,9,11-estratriene acetate) given subcutaneously to female rats increased their growth rate compared with untreated controls ($P < 0.001$).
2. The compound had little androgenic activity.
3. The fractional synthetic and fractional degradative rates of the mixed muscle proteins were smaller in the treated rats than in the control rats.

Generally the male mammal grows faster than the female. However, the male is often more aggressive and difficult to handle in captivity. This and other problems have led to the use of the castrate male in many animal production systems. The presence of testosterone in the male accounts for the comparatively rapid growth rate and for the development of the sexual characteristics. These two properties are termed the anabolic and androgenic characteristics respectively. For intensive animal production systems, it would be ideal if the anabolic characteristics could be retained without the androgenic activity. This ideal has led to be development of a series of synthetic compounds with anabolic activity but without significant androgenic activity. One of the currently used compounds, Trienbolone acetate, is the subject of this communication. Trienbolone acetate implants have been shown to increase markedly the growth rate of heifer beef (Best, 1972; Heitzman & Chan, 1974), veal calves, bulls, castrate male lambs and castrate male pigs (Grandadam, Schield, Jobard, Dreux & Boisson, 1975).

The deposition of protein induced after treatment with steroids has been suggested to be the result of an increase in protein synthesis coupled with a decrease in the rate of protein catabolism (Barragry, 1974). However, most studies have concentrated on isolated tissue preparations observing the production of specific proteins (see for example Florini & Breuer, 1965; Jensen & DeSombre, 1972). The results of the data presented here would indicate that while Trienbolone acetate induced an increase in the rate of growth of female rats, its mode of action was mediated by a reduction in the rate of protein synthesis coupled with a reduction in the rate of protein degradation.

MATERIALS AND METHODS

Specific pathogen-free rats of the Wistar strain (60–80 g initial wt) were used and allowed access to food and water *ad lib*. Female animals were used except for the Herschberger test (Herschberger, Shipley & Meyer, 1953) which requires the use of

castrate males. Rats were randomly distributed to individual metabolism cages. A 12 h light-and-dark cycle was used.

Trienbolone acetate (3-oxo-17-β-hydroxy-4,9,11-estratriene acetate, obtained from Roussel–Uclaf, Paris) was prepared for injection by dissolving 20 mg in 200 μl ethyl acetate. Corn oil was then added to give a final concentration of Trienbolone acetate in the corn oil of 0·8 mg/ml and the mixture rotary evaporated under reduced pressure to remove the ethyl acetate. Rats were injected subcutaneously via the neck skin fold (80 μg Trienbolone acetate/100 g body wt). Control rats were given corn oil similarly treated but without the addition of Trienbolone acetate.

Fractional synthetic rates of mixed tissue proteins were determined by the continuous infusion method described by Waterlow & Stephen (1968) but using L-[U-^{14}C]tyrosine (Garlick & Marshall, 1972). L-[U-^{14}C]tyrosine (Radiochemical Centre, Amersham, Bucks.) diluted to 2·0 μCi/ml saline, specific activity 10 mCi/mmol was infused into the tail vein at a rate of 0·64 ml/h for 6 h. At the end of the infusion, rats were killed by cervical dislocation, tissues frozen in liquid nitrogen and stored at $-20°$ until analysed. L-tyrosine was estimated in the protein-bound fractions by homogenizing the tissue in cold trichloroacetic acid (100 g/l), washing the precipitate in cold trichloroacetic acid solution and then hydrolysing it for 22 h in 6 M-HCl at 110°. The tyrosine content of the hydrolysate was determined using the method of Waalkes & Udenfriend (1957) and the radioactivity in the tyrosine determined by trapping the $^{14}CO_2$ released on treatment of the hydrolysate with tyrosine decarboxylase (Sigma, London) in Hyamine hydroxide (Fisons Scientific, Loughborough).

The intracellular free L-tyrosine content of tissue samples was estimated by homogenizing the tissue (approximately 1 g) in cold picric acid (10 g/l), washing the precipitate twice with cold picric acid (10 g/l) and combining the supernatants. The combined supernatants (25 ml) were passed down an 8 × 0·8 cm column of Dowex 2 × 8, 200–400 mesh resin (Bio-Rad Laboratories, California) followed by 5 ml 0·02 M-HCl, the effluent was collected in a round-bottomed flask, rotary evaporated to dryness at 37° under reduced pressure and taken up in 2·0 ml of distilled water. L-tyrosine and [^{14}C]tyrosine were estimated as for the protein-bound fraction.

Fractional rates of protein degradation were determined using the $Na_2{}^{14}CO_3$ method described by Millward (1970). Each rat (15 in each treatment) received 250 μCi $Na_2{}^{14}CO_3$ (59 mCi/mmol) intraperitoneally. Three rats from each treatment were killed on days 2, 4, 8, 11 and 14 after the injection.

Male rats were castrated 4 d before they were used for the Herschberger test (Herschberger et al. 1953). They were then divided into three groups each of 6 animals. One group received Trienbolone acetate, the other testosterone (British Drug Houses, Poole, Dorset; 80 μg/100 g body wt per d, prepared as per Trienbolone acetate) and the control group just received oil. After 15 d of treatment the animals were killed and the m. levator ani and the ventral prostate glands removed, blotted and weighed.

Table 1. *Effect of Trienbolone acetate on weight gain and tissue weights (measured as a percentage of final body weight) in the rat*

(Results are expressed as means ± SEM)

	Test (n = 6)		Control (n = 6)	
	mean	SEM	mean	SEM
Initial (g)	86·3	1·69	86·9	1·92 N.S.
Final (g)	163·3	3·42	145·2	2·23 ***
Weight gain (g)	77·0	2·59	58·3	1·65 ***
Kidney	0·99	0·031	0·89	0·021***
Spleen	0·21	0·011	0·25	0·012***
Liver	4·00	0·12	4·10	0·09 N.S.
Heart	0·45	0·013	0·44	0·014 N.S.
Uterus	0·18	0·007	0·13	0·01 **

Significance of difference from each group: ** $P < 0.01$; *** $P < 0.001$; N.S. $P > 0.05$.

Table 2. *Results of Herschberger analysis showing the effect of Trienbolone acetate and of testosterone in the rat*

(All values are mean ± SEM (6))

	m. levator ani (mg)	v. prostate (mg)	m.l.a./v.p.*
Control	51·9± 1·99	15·3±1·81	—
Testosterone	98·1± 3·65	132·9±4·22	0·39±0·030
Trienbolone acetate	160·9±10·13	158·3±6·02	0·76±0·046

$$* \text{ m.l.a./v.p.} = \frac{\text{test m levator ani wt.} - \text{control m. levator ani wt}}{\text{test ventral prostate wt} - \text{control ventral prostate wt}}.$$

Significance of difference between m.l.a./v.p. $P < 0.001$.

RESULTS AND DISCUSSION

The treated female rats grew significantly faster than the control rats ($P < 0.001$). The difference in growth rate was apparent within 4 d of commencing the Trienbolone acetate treatment. There were also differences in organ size (Table 1). This increase in growth rate did not appear to be directly related to any androgenic properties the compound may have. An enlargement of the kidney seen on feeding growth promoters has been interpreted as an indication of anabolic activity rather than androgenic activity (Jelinek, Vesela & Valova, 1964). The increase in weight of the uterus is also not consistent with significant androgenic activity (see Lobl & Maenza, 1975).

Results from the Herschberger test (Herschberger *et al.* 1953) which can be used to compare the anabolic activity with the androgenic activity of a compound, also indicated the enhanced anabolic activity of the compound (Table 2).

Results from the continuous infusion experiments indicated that Trienbolone acetate reduced the fractional synthetic rate of the mixed protein of the muscle and of the heart (Table 3). There was also a similar reduction in the fractional degradative rate. In the present study we are assuming that there was an increased rate of protein deposition in the test animals. Trienbolone acetate has been reported to increase

Table 3. *Fractional synthetic rates and fractional degradative rate of mixed proteins in the tissues of the female rat treated with Trienbolone acetate*

(Values are expressed as mean ± SEM)

	Test		Control	
	fractional synthetic rate/d	fractional degradative rate/d	fractional synthetic rate/d	fractional degradative rate/d
(a) 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**	—
(b) Na¹⁴CO₃				
Hind limb muscle	—	0·048±0·001	—	0·060±0·001***

In experiment (a) fractional synthetic rates were determined using the continuous infusion technique with rats previously injected for 21 d with Trienbolone acetate (80 μg/100 g body wt per d) or with corn oil; six rats in each group. In experiment (b) the Na¹⁴CO₃ technique was used to measure fractional degradative rate. Rats were again injected with 80 μg Trienbolone acetate/100 g body wt per d or with corn oil. For details see text. Significant differences between test and control group given as: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

nitrogen retention (Chan, Heitzman & Kitchenham, 1975). The fact that the difference between the measured synthetic and degradative rates in the test and control animals did not quantitatively account for the increased growth of the treated animals is probably a consequence of the errors, both conceptual and analytical, associated with the techniques used. The observed reduction in protein synthetic rate is again not consistent with significant androgenic activity; in males mixed muscle proteins have a faster fractional synthetic rate than in females (Waterlow & Stephen, 1967).

The simultaneous changes in the fractional synthetic and fractional degradative rates of muscle proteins accompanying changes in growth rate have been reported previously (Millward, Garlick, Nnanyelugo & Waterlow, 1976). This reduction in the over-all rate of protein turnover in the treated animals is consistent with the increased feed-conversion efficiency noted in Trienbolone acetate-treated animals (Heitzman & Chan, 1974); the continual turnover of proteins is an energetically expensive process (see for example Buttery & Annison, 1973). In the pig protein turnover has been calculated to account for 17 % of basal metabolic rate (Garlick, Burke & Swick, 1976).

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