Toxicological aspects of anabolic hormone use in meat production

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Hormones, including anabolic steroids, are intimately involved in the determination of body structure and in the day-to-day and, indeed, minute-to-minute, regulation of bodily functions. We are still at the stage of discovering new hormones and of learning more about the mechanisms of action of those discovered earlier.

Any consideration of the use of hormones in meat production logically begins with a close look at the facts concerning hormone secretion in normal human beings. Relevant data are summarized in Table 1 (Levels of endogenous oestrogens in human tissues and secretions), Table 2 (Daily production and plasma levels of oestrogens in humans) and Table 3 (Daily production and serum levels of testosterone). In the interests of clarity, these tables oversimplify the picture by ignoring low levels of production of less active androgens such as androstenedione and epitestosterone.

Volume 21 of the IARC Monograph Series on the evaluation of the carcinogenic risk of chemicals to humans (IARC, 1979) is devoted to a consideration of cancer risk from sex hormones. A priori, one would expect hormones, certainly natural hormones, to be without mutagenic properties. By contrast there are theoretically numerous epigenetic mechanisms by ~~ways in~~ which hormones might ~~theoretically~~ enhance or diminish cancer risk, particularly if they are administered in high dosage over prolonged periods. Examples of possible ways are listed in Table 4

For me it is not in the least surprising that substances that regulate bodily functions can influence cancer risk in either direction and there is no shortage

of examples of enhancement and inhibition of carcinogenesis by natural hormones in laboratory animals. However, without exception, effects on tumour incidence have only been seen where there is manifest disturbance of hormonal status. Similarly, there can be no doubt that both oestrogens and androgens in hormonally-effective levels can influence cancer risk and cancer growth rates in humans. The best known examples of increased risk are the occurrence of vaginal cancer in the daughters of women given DES during pregnancy and the excessive risk of endometrial cancers in women given hormone replacement therapy at the time of the menopause and afterwards. On the other hand, endocrine ablation and hormone administration are widely used by clinicians in the treatment of patients with cancers of certain kinds.

In considering the safety-in-use of anabolic agents in meat production there would seem to me to be 4 important questions: (i) Is the agent without mutagenic activity? (ii) What is the no-hormonal-effect level (NHEL)? (iii) How does the hormonal activity of residues, if any, in meat relate to that of untreated meat? (iv) How does the activity of residues relate to the daily production and circulation of endogenous hormones in humans?

Are hormones mutagenic?

The IARC Working Group whose deliberations resulted in the 1979 volume on 'Sex Hormones' (IARC, 1979) noted the scarcity of mutagenicity studies on sex hormones and concluded that they needed to see the results of additional studies before making a definitive statement on the subject. In the case of natural hormones I feel that this conclusion exhibits not only excessive caution but also an unjustified lack of confidence in the state of existing knowledge of how hormones work. There is abundant evidence that hormones act by determining the spectrum of phenotypic expression and not by altering genotype. If the effect of a hormone on phenotypic expression in a stem cell is irreversible, its daughter will differ qualitatively from those of unexposed stem cells, i.e. hormones can influence cell and tissue differentiation. It is necessary in the design and interpretation of mutagenicity studies on hormones to distinguish between true mutation from permanent fixation of phenotypic expression.

I regard it as exceedingly unlikely that evolution could have proceeded on the basis that natural hormones exhibit genotoxicity. In the case of unnatural substances with hormonal activity, however, the possibility that the agent itself

or a metabolite of it is genotoxic cannot be ruled out on theoretical grounds. In such cases, therefore, I would need to see negative results in intelligently-designed mutagenicity tests before I could be completely satisfied that the agent, at dose levels below the NHEL, was completely safe.

What is the no-hormonal effect level?

There exist a variety of sensitive methods for measuring anabolic and other effects of sex hormones (Suchowsky, 1964). These include measurements of circulating hormones, observations on target tissues, effects on body growth and effect on kidney weight relative to organ weight. By the use of a relevant combination of such tests it is possible with considerable confidence to identify a NHEL.

Do residues from exogenous hormone-treatment add appreciably to those of endogenous hormones?

Hoffmann et al (1976) found levels of between 0.03 and 0.3 ng/g testosterone in adipose tissue from female calves and between 3 and 22 ng/g in that from sexually mature bulls. Earlier the same workers (Hoffmann et al, 1975) reported levels of th order of 200 pg/g oestrone + 17β-oestradiol in muscle and 400-500 pg/g in adipose tissue from calves. These levels increase to about 10-fold towards the end of pregnancy.

At the 1980 meeting in Warsaw, Hendricks (1980) reported residues of levels of 12-15 pg/g 17β-oestradiol in muscle from untreated heifers and steers and levels 3-4 times higher in the liver and kidney of heifers.

It is against the background levels such as these that the significance of residues of exogenous androgens and oestrogens in meat needs to be judged.

Significance of ingested residues

For the purposes of judging whether residues of hormones in meat are within the range that could have hormonal effects on the meat-eater it would be reasonable to compare possible intakes of residues with (i) daily mean testosterone production in the pre-menopausal non-pregnant woman (i.e. about 0.3 mg see Table 3) in the case of an androgen and (ii) daily mean oestrogen production in the normal adult male (= 70 µg, see Table 2) in the case of an oestrogen. On commonsense grounds I would judge intakes from meat equivalent in hormonal activity to 1/100th of

of 0.3 mg testosterone or of 70 μg 17β-oestradiol to be negligible.

Trenbolone acetate

Since I have been, to a limited extent, involved in the toxicological assessment of trenbolone acetate, it is convenient for me to apply the above principles to this particular agent as an example. Trenbolone acetate is a synthetic steroid closely related structurally to both testosterone and oestradiol. A priori, I would not expect it to exhibit electrophilic properties or to give rise to metabolites with such properties. The fact that it gave negative results in the Ames' test are consistent with this expectation.

Long-term feeding tests in rats and mice have been conducted. In mice the lowest dietary level tested was 0.5 ppm. This marginally enhanced growth rate in females and cannot therefore be regarded as a NHEL in this species. The results of the mouse test are summarized in Tables 5 and 6. The statistical significance of the excess liver tumours shown in the tables persisted after account was taken of survival difference. In rats, (Tables 7 and 8), at the highest two dose levels, TBA treatment led to improved survival and reduced incidences of pituitary and mammary tumours in females. A barely statistically significant excess of pancreatic islet-cell tumours in the top dose female group might have been due to a spurious absence of such tumours in corresponding controls, or it might have been an indirect manifestation of hormonal disturbance (e.g. excessive output of growth hormone by the pituitary gland).

All the effects depicted in Tables 7 to 10, including the excess of liver tumours in the mice and the reduced incidences of pituitary and mammary tumours in the rats are attributable to the known hormonal effects of TBA. No evidence emerged from these studies of any effect that would lead me to suspect TBA of carcinogenicity because of genotoxic activity.

Comparisons of the incidences of liver tumours in overfed and diet-restricted mice and of pituitary and mammary tumours in overfed and diet-restricted rats illustrate that big differences in the incidences of such tumours and in survival can be brought about without changing the level of exposure to any genotoxic carcinogen (Tables 9 and 10). Gellatly (1975) found that liver tumour incidence in mice increased 9-fold when he doubled the concentration of ground nut oil in a semi-synthetic diet (Table 11).

Under conditions of recommended use, the residues of TBA in meat amount to about 0.3 µg/g. This is equivalent to the top end of the range of concentration of endogenous testosterone present in adipose tissue from calves and is only between 1/75th and 1/10th of that in the adipose tissue of the mature bull (vide supra).

According to Ross (1981) 0.1 ppm TBA in the diet is a NHEL in the pig. A 60kg human consuming 500 g/d meat derived from a TBA-treated animal would consume daily a maximum of about 150 ng of TBA. Compared with the estimated daily production of testosterone by a premenopausal non-pregnant woman (i.e. 300 µg), this intake of 1/2000th of that amount, must be deemed negligible, unless TBA is androgenically far more potent than testosterone, which it is not. In fact these calculations are conservative since TBA is relatively heat-labile and therefore subject to destruction during cooking.

As a final check of the safety-in-use of TBA in meat production, in an experiment lasting altogether 114 weeks, rats of 3 successive generations were fed on a diet containing meat from steers given up to 25 times the recommended exposure to TBA (Kaemmerer, et al, 1978). In this relais study no hormonal or other toxic effects of any kind were seen.

Conclusion

In this paper I have discussed the principles involved in assessing whether residues from hormones used in meat production are likely to constitute a cancer hazard for the meat-eater. Applying these principles to the anabolic steroid, Trenbolone acetate (TBA), I concluded that its use presents no cancer hazard.

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Table 1: Levels of endogenous oestrogens in human tissues and secretions

Tissue	Hormone	Level	Reference
Ovary	Oestrone	0.1 - 0.16 µg/g	Mahesh & Greenblatt, 1964
	17β-oestradiol	0.25 - 0.41 µg/g	
Placenta	Oestrone	0.41 µg/g	Schmidt-Elmendorff, 1961
	17β-oestradiol	0.1 - 0.18 µg/g	
Semen	Oestrone	0.06 ng/ml	Diezfalusy, 1954
	17β-oestradiol	0.01 ng/ml	
	Oestriol	0.03 ng/ml	

Table 2: Daily production and plasma levels of oestrogens in humans

Subject	Mean daily production of 17β-oestradiol (μg/d)	Combined levels of 17β-oestradiol, oestrone & oestriol in plasma (ng/ml)	Reference
Women - start of menstrual cycle	35 - 100	60	Roy et al, 1965
Women - mid cycle	up to 300	100	"
Women - end of pregnancy	5000	6000	Aitken & Preedy
Men - aged 21-37	70	30	Pochi et al, 1965

Table 3: Daily production and serum levels of testosterone

Sex	Testosterone production	Serum level	References
	mg/d	ng/ml	
Male	7	7	Horton & Tait, 1966 Riondel et al, 1963
Female			
- non-pregnant	0.3	0.4	Lloyd et al, 1966
- late pregnancy	-	9	Meeker, C.I., 1966 Bardin & Lipsett, 1967

Table 4

Possible epigenetic mechanisms whereby hormones may enhance or inhibit cancer development

1. Structural development change (daughters of DES-treated women)

2. Altering metabolic activation of pro-carcinogens

3. Immunosuppression

4. Activating oncogenic virus (mouse mammary tumour virus)

5. Stimulating or inhibiting DNA synthesis and mitosis necessary for fixation of transformed state.

6. Permanent damage to negative feed-back control mechanism with consequential output of another hormone

Table 5

Effects of TBA in male mice (52 per group)

ppm TBA in diet	0	0.5	1	10	100
% survival to 96 weeks	44	35	42	37	25
% malignant tumours - all sites	38	33	38	44	34
% liver tumours +	17	27	27	33*	37**

+ All percentages are within the range of historical controls

* $p < 0.05$ ** $p < 0.01$

Table 6

Effects of TBA in female mice (52 per group)

ppm TBA in diet	0	0.5	1	10	100
% survival to 96 weeks	21	25	27	35	38
% malignant tumours - all sites	65	44*	44*	47	46
% liver tumours +	2	1	3	5	8**

+ All percentages within range of historical controls

* $p < 0.05$ ** $p < 0.01$

Table 7

Effects of TBA in male rats (50 per group)

ppm TBA in diet	0	0.5	1	4	16	50
% survival to 110 weeks	26	24	22	22	22	38
% liver tumours	0	6	2	4	4	1
% pituitary tumours	28	30	26	30	28	24
% islet cell tumours of pancreas	10	12	12	6	2	20

Table 8

Effects of TBA in female rats (50 per group)

ppm TBA in diet	0	0.5	1	4	16	50
% survival to 110 weeks	32	16	22	32	64	56
% liver tumours	0	0	2	0	0	2
% pituitary tumours	34	60	50	44	36	20
% mammary fibroadenomas	74	70	76	60	48	4
% islet cell tumours of the pancreas	0	4	4	8	6	12

Table 9

Effect of simple dietary restriction on tumour incidence in mice†

no. of mice which developed tumours at any time during the study. There were 160 mice of each sex in each group

	Males		Females	
Feeding regimen ...	*Ad lib.*	Restricted to 75% of *ad lib.*	*Ad lib.*	Restricted to 75% of *ad lib.*
Type of tumour				
Lung	30	19•	24	8••
Liver	47	12•••	7	1•
Lymphoma	4	1	11	4•
Other	8	4	12	4•
Any tumour at any site	71	36•••	50	17••
Any malignant tumour	17	7•	23	7••

•$P<0.05$, ••$P<0.01$, •••$P<0.001$.

†Conybeare, 1980.

Table 10

Effect of dietary restriction on incidence of pituitary and mammary tumours in rats[+]

	Males		Females	
Feeding regimen ...	*Ad lib.*	Restricted	*Ad lib.*	Restricted
Rats with pituitary tumours (%)	32	0•••	66	39••
Rats with mammary tumours (%)	0	0	34	6•••

••$P<0.01$, •••$P<0.001$.

+ from Tucker, 1979

Table 11

Dietary fat and liver tumours in C57BL female mice•

	Mice with liver tumours (%)	
	Benign or malignant	Malignant
SS diet with 5% GNO	8	1
SS diet with 10% GNO	43	9

GNO, groundnut oil.
•Gellatly, 1975.