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Results of long-term rodent studies with special reference to cancer risks and hormonal no-effect levels

F.J.C. ROE*

THE NATURE OF CARCINOGENESIS

Hormones generally present difficulties for Regulatory Authorities, whose job it is to protect the general public from cancer risks associated with foods or drugs. It is mainly for this reason that it is proving so difficult to persuade such authorities to make any clear decisions or recommendations in this area. At the root of the matter is a lack of understanding of the nature of carcinogenesis combined in some cases, with serious misconceptions of the process.

According to the simplest definition, '*a carcinogen is any agent that increases the age-standardized risk of cancer development in a defined population of laboratory animals or humans*'. Although this definition cannot be faulted, it is nevertheless not very helpful when it comes to Regulation, because all kinds of rather innocent substances can inadvertently fulfill the definition of 'carcinogen' in one or other set of circumstances. For instance, excess food is highly carcinogenic in laboratory rats and mice according to this definition of carcinogenicity.

It is now widely agreed that two kinds of activity are, or may be, involved in the carcinogenic process. The first of these, which is sometimes referred to as *tumour initiation*, is thought to involve an alteration of the genetic components of the cell - either an alteration in the DNA or a particular gene (*gene mutation*) or a change on a much bigger scale involving whole chromosomes (*clastogenesis* or *chromosomal aberration*). The second kind of activity, often referred to as *tumour promotion*, is thought of as following tumour initiation in time and as involving the stimulation of cells altered by the initiating process to proliferate and form visible neoplasms. This two-stage concept is clearly over-simplistic, particularly since it brings together under the heading 'tumour promoters' a very mixed bag of possible mechanisms which have little or nothing in common.

* Consultant in Toxicology and Adviser in Experimental Pathology and Cancer Research, 19 Marryat road, Wimbledon Common London, SW 19 5BB, Great Britain

Many hormones in high doses have been shown to enhance cancer risk under one or other set of circumstances. According to our simplest definition 17β-estradiol, testosterone and progesterone are all carcinogens, since all these agents can easily be shown to enhance cancer risk under certain circumstances.

Table I. TYPICAL PERFORMANCE OF A HORMONE IN TESTS FOR MUTAGENICITY, CLASTOGENICITY AND CELL TRANSFORMATION

Ames test	-
Covalent binding to DNA	-
Non-covalent binding	+
Cytogenetics - *in vitro*	-
- *in vivo*	-
Mammalian cell mutation	±

Margaret Richold, who spoke earlier, has succinctly reviewed what happens when sex hormones and other hormones are submitted to various tests for mutagenicity and clastogenicity. Characteristically, they give negative results in the Ames test and in tests for clastogenicity, positive results in tests for cell-transformation and equivocal results in the L5178Y mouse lymphoma assay. According to Ashby (1982) this pattern seems to be the hallmark of hormones.

Taking the mutagenicity and carcinogenicity data together, the general position is that sex hormones, both natural and synthetic, are non-genotoxic but nevertheless may increase the risk of development of certain forms of neoplasia when given to animals in doses that manifestly affect hormonal status. This has led many observers to class them as *tumour promoting* agents as distinct from *tumour initiating* agents or *complete carcinogens*.

It is interesting that recent research has shown that the classical tumour-promoter - namely, the phorbol ester known as TPA - has been shown to produce its effects by a hormone - like action. It reacts with cell epidermal cell surface reactors which respond physiologically to epidermal cell growth factor - recently discovered peptide hormone.

DOSE-RESPONSE AND THRESHOLDS

In the case of carcinogens which are manifestly genotoxic, conventional prudence dictates that one should assume that there is no threshold level of exposure that is wholly without cancer risk. There are grounds for suspecting that this approach is in some cases overcautious and that the intact organisms are well-equipped to cope with exposure to low doses of some environmental mutagens without coming to harm. However, since proof of this is usually elusive, most observers advise that exposure to genotoxic agents should be minimized. Far less worry attaches to non-genotoxic tumour promoting agents, and therefore to hormones, for which there are not even theoretical reasons for believing that thresholds may not exist.

For such agents a primary objective of animal tests should be, not to see whether tumours arise when doses are pushed up to levels that are only barely tolerated, but to identify levels below which there is no measurable effect on any parameter which reflects hormonal status.

The fact is that, in a vast literature concerning the affects of hormones on cancer incidence, there are no clear and unequivocal examples of an adverse effect on tumour incidence in the absence of a previously obvious effect on hormonal status. It is therefore reasonable to assume that humans would be at no cancer risk from exposure to hormones in meat under conditions where residue levels are below those associated with hormonal effects.

Against this brief background we may look at the data from toxicity and carcinogenicity tests on trenbolone acetate (TBA) and zeranol (Z).

TRENBOLONE ACETATE (TBA)

Theoretically, the most relevant data for TBA are those from a relay toxicity study in which Sprague Dawley rats were fed on diets containing meat from treated heifers, since in this study the animals were exposed to both TBA and to its metabolites. In this small study, rats were exposed to meat containing over 200 times the TBA residues normally found in meat from treated animals, and no remarkable effects were seen over 3 generations. However, the animals were not observed for a long enough part of their life-span to exclude a possible effect on tumour incidence.

In a life-span carcinogenicity study in rats carried out at the Huntingdon Research Centre, groups of 50 males and females were exposed, starting in utero, to 0, 0.5, 1.0, 4.0, 16.0 or 50 ppm TBA in the diet. Enhancement of body growth and effects on the genitalia were seen in response to 1 ppm and higher levels. The only adverse

effect on tumour incidence was on pancreatic islet cell tumours in the top dose group and this was more than offset by a marked reduction in mammary tumour incidence in the same group. (Table II)

Table II. MAMMARY FIBROADENOMAS AND PANCREATIC ISLET-CELL TUMOURS IN TBA-TREATED RATS

TBA in diet (ppm)	0	0.5	1	4	16	50
% mammary fibroadenomas	74	70	76	60	48	4**
% with islet-cell tumour						
- males	10	12	12	6	2	20
- females	0	4	4	8	6	12*

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

It is well known from numerous studies on animals that pancreatic islet cells proliferate in response to growth hormone. Several workers (5, 7, 8, 6, 4, 9) reported the induction of an insulin-secreting pancreatic neoplam in hamsters with testosterone. It is thus not surprising that an agent which stimulates growth and which is structurally related to testosterone should, in high doses, give rise to proliferative changes in islet tissue.

In a 2 year study in Swiss mice also carried out at the Huntingdon Research Centre, groups of 52 males and females were exposed to 0, 0.5, 1.0, 10 or 100 ppm TBA in the diet. Enhancement of growth was seen at all dose levels in females. Liver tumour incidence was increased in males on the 10 ppm diet and in both sexes on the 100

Table III. MAIN EFFECTS OF TBA IN MALE MICE

TBA in diet (ppm)	0	0.5	1	10	100
% survival to 96 weeks	44	35	42	37	25
% liver tumours	17	27	27	33*	37**
% one or more malignant rumours at any site	38	33	38	44	35

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

ppm diet. The incidence of malignant tumours of all sites combined was reduced by TBA at all dose levels in females despite a dose-related improvement in survival (Tables III and IV).

Table IV. MAIN EFFECTS OF TBA IN FEMALE MICE

TBA in diet (ppm)	0	0.5	1	10	100
% survival to 96 weeks	21	25	27	33	38
% liver tumours	2	1	3	5	15**
% malignant tumours at any site	65	44*	44*	47	46

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

Apart from Dr. Taylor's evidence of the effect of methyltestosterone on liver tumour incidence, there is a considerable body of published evidence that the male sex-hormone favours liver tumorigenesis in mice. Andervont (2) found that castration reduced a spontaneous liver cell incidence from 34% to 11% in C3H male mice and Agnew and Gardner's (1) experiments also pointed to the enhancement of liver tumour incidence in mice by androgens.

Characteristically genotoxic carcinogens such as dimethylnitrosamine give rise to a variety of different kinds of liver tumour including tumours of the bile duct and vascular components as well as of the parenchymal cells. The extra tumours seen in the TBA treated mice were all of parenchymal cell origin and indistinguishable from those occurring in the untreated control group. Almost certainly, therefore, this effect of TBA on liver tumour incidence in mice was secondary to the known androgenic properties of TBA.

ZERANOL

Interest in the properties of zeranol at first related to its potential use as an oral contraceptive. For this reason there exist for this agent data from studies in which female dogs were exposed to it for up to 7 years and female rhesus monkeys for up to 10 years. The studies were not designed to identify a no-hormone-effect level. Consequently, the dose levels studied in all three species were well within the range expected to have hormonal effects. (Table V).

The hormonal effects encountered were all of kinds expected from prolonged exposure to an estrogen, including squamous metaplasia and dysplasia of the uterine cervix in the monkeys. No excess of any form of cancer was recorded.

Table V. ZERANOL: CARCINOGENICITY AND LONG-TERM TOXICITY DATA

Species	Duration	Sexes	Doses (mg/kg/d)
Rat	2 years	Both	0, 0.8, 6.4, 0.1, 20
Dog	7 years	Female	0, 15, 37.5 (21 days on, 7 days off)
Monkey (Rhesus)	10 years	Female	0, 15, 77 (21 days on, 7 days off)

These data, viewed together with the results of mutagenicity tests on zeranol as reported earlier by Dr. Parekh, suggest that exposure to residues of zeranol at dose levels below those that exhibit hormonal effects would pose no cancer risk to humans.

CONCLUSION

1. Hormones generally pose no cancer risk where exposure is to levels below those required for detectable hormonal activity.

2. Mutagenicity and carcinogenicity test data for TBA and zeranol suggest that these agents and their metabolites are neither mutagenic nor clastogenic and that they would only influence cancer risk - either increase it or decrease it - if there was exposure to hormonally effective levels.

3. Therefore, in judging whether it is safe to use TBA and zeranol as anabolic agents in meat production the emphasis needs to be on making sure that any residues of these agents in meat are below the levels that could have any hormonal effect on the meat-eater.

4. It is clear to me from the data already discussed at this symposium that the levels of active residues of either agent in meat are almost certainly several orders of magnitude below those that could be expected to have any hormonal effect in humans. However, in order to put a more precise figure on the safety

margin, it is necessary to establish no-hormone-effect levels for the two substances. The next three speakers review relevant data in this area.

SUMMARY

RESULTS OF LONG-TERM RODENT STUDIES WITH SPECIAL REFERENCE TO CANCER RISKS AND NO-HORMONE LEVELS

F.J.C. ROE

The results of long-term studies on trenbolone acetate (TBA) and zeranol (Z) are summarized in the light of long-term toxicity data for endogenously produced oestrogens and androgens. An increase in liver tumour incidence in mice exposed to 10 or 100 ppm TBA in the diet was not unexpected in view of the well-documented relationship between androgenic status and liver tumour risk in mice and evidence that testosterone has a similar effect. An equivocal enhancement of islet-cell tumours of the pancreas in rats exposed to a diet containing 50 ppm TBA may, if real, be attributable to an effect of growth hormone on pancreactic islet tissue. In the interpretation of these data for TBA, two facts are of paramount importance : there was no evidence of enhancement of cancer risk at any site that did not also respond to the known hormonal activity of the agent and enhancement of tumour risk was only seen where there was exposure to levels of TBA that both produced hormonal effects and were greatly in excess of human exposure levels resulting from the use of this agent in meat production. In rats, 0.5 ppm was both a no-toxic effect and a no-hormonal effect level, exposure to 50 ppm TBA was associated with a significant reduction in the incidence of mammary tumours in female rats, and 16 ppm was a no-effect level for enhancement of pancreatic islet cell tumour incidence. In mice, 0.5 ppm was seemingly close to a no-hormone-effect level. The only effects seen in response to this level in the diet were slight growth enhancement in females and a significant reduction in the incidence of maligant neoplasms of all sites combined. These data from long-term in vivo studies, viewed together with the extensive negative data from tests for mutagenicity and clastogenicity indicate that humans exposed to residues of TBA below the levels needed to produce hormonal effects would be at no increased risk of developing any form of cancer.

The data so far available from long-term rodent studies on Z are less extensive. No enhancement of tumour risk at any site was seen in a 2-year rat study involving exposure to dietary levels in the range of 0.8 to 20 mg/Kg/d, however, histopathological changes attributable to

the hormonal effects of Z were seen at all dose levels including the lowest. Again, however, data from mutagenicity tests have been consistently negative. Taking into account not only these two sets of data but also those from tests of other kinds and in other species, there are sound reasons for concluding that Z poses no carcinogenic risk where exposure is to levels below those associated with evidence of hormonal activity.

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