HORMONAL AND NUTRITIONAL FACTORS IN CARCINOGENESIS: AN OVERVIEW

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ABSTRACT

The subject of direct and indirect tumour induction by hormones as manifestations of non-genotoxic carcinogenesis is briefly reviewed. It is suggested that in the case of hormonal carcinogenesis the risk of mutation is increased by unremitting over-stimulation of endocrine and hormone-responsive tissues and that this is a late rather than an early stage in hormonal carcinogenesis.

Most carcinogenicity studies in laboratory rodents are conducted under conditions of overfeeding. In rats overfeeding predisposes on the one hand to progressive nephropathy and other non-neoplastic diseases and on the other hand to hormonal disturbances and neoplasia. Certain inter-relationships between overfeeding, nephropathy, phaeochromocytoma and other forms of neoplasia and calcium metabolism are discussed. Finally, the inverse relationship between reduced prolactin and oestrogen dominance is briefly considered.

Hormonal Carcinogenesis

Introduction

Traditionally, quite different kinds of investigator have been interested in the role of hormones in carcinogenesis as distinct from non-hormones. Hormonal carcinogenesis has been a scientific playground for biologists rather than for chemists and biochemists. Two developments in the last two decades have begun to bring these and other disciplines together: the first is the rapid growth of molecular biological technology and the second is the burgeoning interest in peptide hormones and homeostatic control mechanisms.

It should not be so, but alas it is so, that fashions in science follow the development of methods rather than the reverse. Mainly for this reason, we have endured, and are still enduring, a fashion based on the belief that genotoxicity is temporally the first, the most important, and possibly the only, step in carcinogenesis. Many investigators still design their studies on the basis of this belief and innumerable scientific conferences have been organised on the assumption that it is a true creed. Nevertheless, as the title of the present conference illustrates, the faith of some is beginning to falter. The question now is "How far will this defection go?" Obviously it will continue to be possible for those whose life's research involves experimenting with potent mutagenic carcinogens to continue as if non-genotoxic carcinogens did not exist. However, for those of us grappling with real-life problems, including the prevention of cancer in man and the safety evaluation of an ever increasing number of carcinogenic but not mutagenic chemicals, a growing question is: "To what extent is disturbance of hormonal status responsible for enhanced cancer risk?"

The picture has been distorted by over-concentration on synthetic hormones, particularly diethylstilboestrol (DES), and inadequate study of natural hormones. The simple fact is that natural hormones can, under certain circumstances, give rise to cancer in the absence of known exposure to any exogenous genotoxin. This fact is illustrated by the classical experiments of Biskind and Biskind (1944) and of Gardner (1948). In these experiments both ovaries were removed, from a rat or a mouse, and one of them was implanted back into the spleen of the animal from which it had been removed. The ovary in its natural position in the body, in response to gonadotropin secreted by the pituitary, produces oestrogens which find their way, via the systemic circulation, back to the pituitary. There they inhibit the production of excessive gonadotropin. By this negative feedback mechanism the outputs of both gonadotropin by the pituitary and of oestrogen by the ovary are controlled. But the ovary residing unnaturally within the spleen produces oestrogens which pass directly to the liver via the splenic vein. In the liver they are largely inactivated during the first pass with the result that the pituitary does not get the message to cut down its production of gonadotropin. The eventual results are, first, that the pituitary becomes hyperplastic and then neoplastic. Secondly, the ovary within the spleen becomes hyperplastic and then neoplastic and thirdly, the liver gets very big and becomes filled with blood (peliosis hepatis) because it cannot cope with the job of inactivating so much oestrogen. In the light of evidence such as this, it is difficult to deny that natural hormones cannot act as carcinogens. For me this evidence is far more persuasive than that for the daughters of women treated with DES during pregnancy who exhibit an increased risk of cancer of the lower genital tract. Not only is DES not a natural hormone, but the indications are that in this case cancer risk is increased by an indirect mechanism dependent on

developmental abnormalities induced by oestrogens during foetal life rather than by a direct effect on normal structures.

An overview of the literature for endocrine glands and hormone-sensitive tissues leaves no doubt in my mind that, by any common sense definition of the term 'carcinogen', several natural hormones are carcinogens. This is true for 17β-oestradiol, it is true for testosterone, it is true for thyroid-stimulating hormone (TSH) and true for prolactin, etc.

Do hormones really lack genotoxic potential?

If one undertakes a large enough number and variety of tests for mutagenic and/or clastogenic activity, one inevitably obtains a few equivocal or spuriously positive results. Those who cannot countenance that there can exist non-genotoxic carcinogens are prepared to go to almost any length to prove that a carcinogenic hormone is, in fact, genotoxic. I see no need to try to force the facts to fit the theory in this way, and favour far more the simple view recently put forward by Dr. John Ashby (1986) that genotoxicity testing should be confined to the use of a limited number of well-validated sensitive tests (specifically, an *in vitro* Salmonella test for mutation, an *in vitro* test for chromosomal aberration in mammalian cells, an *in vivo* mouse bone marrow assay and a liver test for unscheduled DNA synthesis). If these give negative results, then one should accept that any enhancement of cancer incidence seen *in vivo* is brought about indirectly by an essentially non-genotoxic mechanism rather than directly by so far undetected genotoxicity of the test substance.

As far as I know, all natural hormones come out negative in Ashby's short list of tests. I see no point in exploring an unending list of other tests to see if positive results can be obtained and I see no justification in extrapolating from

results obtained with unnatural hormones such as DES to natural hormones such as 17β-oestradiol.

The fact that some hormones have been found to transform cells in vitro, to my mind, merely confirms that cell-transformation is not a reliable index of mutation.

How much human cancer is hormonal in origin?

A common sense interpretation of the Cancer Registry data collected by Waterhouse (1974) suggests that rather less than 10% of cancers in men, but as many as 35% of cancers in women primarily involve hormones (see Table 1). However, this interpretation ignores the fact that hormone-induced cancers sometimes pop up where you do not expect them. Thus, exposure of the male hamster to oestrogens predisposes to the development of renal tumours even though the kidney is not normally regarded as a target for oestrogen activity. More serious, however, is the fact that the estimates shown in Table 1 are based on a rather limited and old-fashioned concept of the term 'hormone'. If the term is extended to include regulatory peptides and the rapidly increasing number of endogenous messenger substances involved in homeostasis then it can be envisaged that hormones are directly implicated in the causation of a large proportion of human cancers.

How much cancer in laboratory animals is hormonal in origin?

Previous speakers (Conybeare pp. - , Newberne pp. - , and Turnbull pp. -) have discussed various ways in which tumour incidences in long-term animal studies may be influenced by nutritional factors. Earlier (Roe, 1981) I pointed out that in rats, most of the tumours associated with overnutrition are of endocrine glands or hormone-responsive tissues. It is now clear that overfeeding causes a variety of hormonal disturbances in rats as indicated by changes in levels

of circulating hormones and irregularity of oestrous cycling (see Conybeare pp. -). In mice, overfeeding can markedly enhance tumour incidence but the sites mostly affected are those at which neoplasia is normally most common in untreated animals of this species, (i.e. the lung, the liver and the lympho-reticular system) and not endocrine glands.

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There are no reliable tumour incidence data for 'normal' rats and mice because normality is indefinable. Clearly the incidences of endocrine/hormonal tumours shown in Table 2 for untreated control rats in a two-year carcinogenicity study reported by Kociba et al (1979) are not those for "normal" animals and there are no reliable tumour incidence data for rats in the wild. The best that can be said is that the incidences of endocrine/hormonal tumours in rats can be reduced markedly by the avoidance of overnutrition (see Table 3 derived from Tucker,1979).

Whether it is really possible to devise conditions in which rats and mice can be maintained in laboratories without developing endocrine disturbances which predispose to cancer is not presently clear. If such conditions exist, they will obviously have to involve dietary restraint. Less certain is whether animals will have to be provided with facilities for exercise and sexual fulfilment. The preliminary data which Conybeare presented earlier in this meeting suggest that the excessive exercise provides no benefit in terms of tumour risk. Whether moderate exercise would do so remains uninvestigated. Data reported in a paper by Pickering & Pickering (1984) suggest that, allowing ad libitum-fed female rats to mate slightly delayed the time at which they developed pituitary tumours. However, the beneficial effect was quite trivial.

An overview of the presently available data, including those reported in numerous papers by the late Morris Ross and his colleagues (e.g. Ross et al, 1982) is that, in rats, dietary practices early in life have profound effects both on

future body size and on propensity to develop endocrine disturbances and tumours. According to John Higginson (1986, personal communication) the same is probably true of humans. On the other hand, Weindruch and Walford (1982) reported that, in mice, dietary restraint begun when animals were 1 year old was still beneficial in terms of tumour risk.

The production of tumours by exogenous hormones involving direct interference with endocrine status

The literature is so full of examples of the production of tumours both in rodents and in man by the administration of exogenous sex hormones, that there is no need for me to review this subject here (see IARC, 1974 and 1979).

Similarly, there are many well-known examples of the production of tumours by agents which interfere with the functioning of endocrine glands. Later in this meeting, Professor Zbinden will be discussing such mechanisms in relation to thyroid (e.g. exposure to goitrogens or iodine) carcinogenesis and Dr. Howatson will be talking about the regulatory peptide-mediated production of tumours of the exocrine pancreas by trypsin-inhibitors present in raw soy flour.

Indirect hormonal carcinogenesis

In Table 4, I have listed 4 examples of indirect hormonal carcinogenesis. The first of these - the enhancement of thyroid follicular neoplasia by liver enzyme inducers - will be discussed by Professor Zbinden.

My second example is drawn from the field of metal carcinogenesis. If rats are exposed acutely to cadmium salts in sufficient dosage, their testes undergo complete atrophy. Whether this is a direct effect of the metal on cells of the

seminiferous tubules or an indirect consequence of cadmium-induced blockage of testicular blood vessels (ischaemia) is uncertain (Roe et al, 1964; Gunn et al, 1963). In the wake of the testicular atrophy, castration cells appear in the pituitary. These cells secrete follicle-stimulating hormone (FSH) and the increased secretion of this hormone leads to hyperplasia and, eventually, neoplasia of the interstitial cells (Leydig cells) of the testis. It is to be presumed that initial atrophy of the testis interrupts a negative feedback control mechanism for FSH production.

Elsewhere, Albert Bär and I (Roe & Bär, 1985) reviewed scattered data pointing to a relationship between disturbance of calcium metabolism and increased incidence of adrenal medullary hyperplasia and neoplasia (phaeochromocytoma) in rats. The archetype for this association is the reponse of rats to diets containing high levels of lactose. The feeding of such diets is associated with markedly increased calcium absorption and excretion and it seems to be this increased calcium throughput which leads to stimulation of the adrenal medulla. But the adrenal is not the only target site for indirect hormonal effects by dietary lactose in rats. As shown in Table 5 (which is based on my own reading of sections derived from a study conducted at TNO/CNO (Zeist, Netherlands, and supported by the EEC-Commission) a diet containing 20% lactose not only significantly increased the incidence of proliferative and neoplastic changes in the adrenal medulla but also significantly increased the incidence of Leydig-cell tumours and significantly reduced the incidence of islet-cell tumours of the pancreas.

Whether any of these effects would have been seen under conditions in which control animals were more or less free from such neoplasms is doubtful. The problem is that virtually all carcinogenicity tests in rodents are conducted under conditions of overfeeding and although there is increasing awareness that

background tumour incidences could be cut down by dietary restraint, no one has the nerve to risk moving away from the present system for fear that Regulatory Authorities would not accept negative results obtained from a carcinogenicity test performed in a new way. The fact is that overfeeding predisposes both to Leydig-cell tumour development (Yu et al, 1982) and to adrenal medullary tumour development (vide infra).

In my opinion, it is wholly wrong to ignore, as some Regulatory Authorities tend to do, beneficial effects of exposure to a test agent on tumour incidence. Such information demands an explanation as much as an adverse effect. The fact is that in the carcinogenicity bioassays conducted by the National Toxicology Program in the USA, beneficial effects are approximately as common as adverse effects (Haseman, 1983) and endocrine glands and hormone responsive sites figure largely in the lists of effects in both directions.

The bromocriptine story

In their test of the bromocriptine for chronic toxicity and carcinogenicity in rats, Richardson and Lunginbuehl (1983) noted decreased incidence/severity of nephropathy and pituitary tumours but increased incidence of endometrial hyperplasia and neoplasia (Table 6). They suggested that, prolactin is a risk factor to nephropathy in rats, and that, if prolactin secretion by the pituitary is suppressed by bromocriptine, animals go into a state of "oestrogen dominance". In looking through data from carcinogenicity studies on various drugs and other chemicals, I have noticed a tendency for nephropathy, mammary tumours and pituitary tumours to be lower in incidence when the incidence of uterine changes are increased and vice versa. However, I find it difficult to accept that conditions could not be devised in which all

these manifestations of toxicity and hormonal change in female rats are avoided.

The consequences of overfeeding in terms of nephropathy and hormonal neoplasia in rats

Diet restraint reduces the incidence/severity of progressive nephropathy in rats. Numerous investigators have reported this. Some of our own data that illustrate this association are summarized in Table 7.

In a recently conducted carcinogenicity study on a drug, there appeared to be a dose related increase in adrenal medullary tumour incidence. I reviewed, in two separate exercises, blindly and in random order, all the kidney sections - grading them for severity of nephropathy - and all the adrenal sections - grading them for adrenal medullary proliferative disease (i.e. hyerplasia and/or neoplasia). I found a highly significant correlation between incidence/severity of the 2 conditions, (Table 8). It transpired that this correlation was true in each treatment group separately and in the control group. Furthermore, after standardizing for nephropathy grade, treatment with the drug was wholly without influence on the incidence/severity of adrenal medullary pathology.

Of course, the demonstration of an association does not establish causality. Nevertheless, it is tempting to conclude that moderate or severe nephropathy predisposes to adrenal medullary proliferative disease. Furthermore since advanced nephropathy interferes with calcium homeostasis which predisposes to parathyroid hyperplasia and neoplasia, it seems plausible that the association between the kidney and adrenal changes was mediated by interference with calcium homeostasis.

Obviously, further research in this area is necessary. However, it does seem that we may be getting nearer to an

understanding of at least one form of overfeeding-associated endocrine neoplasia in the rat.

Conclusions

I have drawn two main conclusions from my survey of hormonal carcinogenesis. The first is that I see no justification for thinking of hormones as "tumour promoters" that only produce tumours after prior exposure of tissues to an initiator. It is patently clear that hormones can produce tumours without prior exposure to a genotoxin. Indeed the position seems to be quite round the other way. Where hormones produce tumours, the mutations involved seem to occur as a second stage and not a first one. In other words, hormones seem to increase the risk of mutation within responsive tissues, possibly by increasing the endogenous generation of electrophiles to a point which exceeds the capacity for repair. Secondly, I remain convinced that it is nothing short of madness to continue to conduct carcinogenicity tests in overfed rats and mice, such that untreated control animals develop multiple tumours secondary to overnutrition-associated endocrine disturbance.

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Table 1

Proportion of human cancers in the UK that are possibly primarily of hormonal origin

(Based on Cancer Registry data - Waterhouse, 1974)

Cancer site	Rate per 100,000 living - all ages Male	Female
All sites	305.6	281.3
Endocrine/hormone sensitive sites		
Breast	0.5	69.1
Endometrium		12.4
Ovary		14.3
Prostate	21.5	
Testis	2.3	
Endocrine (excluding pancreas and gonads)	1.4	2.7
% Endocrine/hormonal	8.4	35.0

Table 2

Certain hormone-associated neoplasms (%) in ad libitum-fed untreated control Sprague Dawley Rats observed for up to 26 months (86/sex)

		Male	Female
Pituitary		31	63
Adrenal	- cortex	2	7
	- medulla	51	8
Thyroid	- C-cell	8	8
Pancreas	- exocrine	33	0
	- endocrine	16	9
Gonad	- testis	7	-
	- ovary	-	5
Mammary Gland	- fibroadenoma		
	- adenoma	5	>76
	- other		

(from Kociba et al, 1979)

Table 3

Effect of 20% food restriction on % survival to 2 years and % tumour incidence in rats
(Tucker, 1979)

	Ad lib	20% restricted
Males		
Survival to 2 years	72	88
One or more tumours at any site	66	24---
>1 tumour at any site	22	2--
Pituitary	32	0---
Females		
Survival to 2 years	68	90
One or more tumours at any site	82	57--
>1 tumour at any site	26	10-
Pituitary	66	39--
Mammary - benign or malignant	34	6---
- malignant	12	2-

\- = $p<0.05$
-- = $p<0.01$
--- = $p<0.001$

Table 4: Examples of indirect hormonal carcinogenesis

1. Liver enzyme induction leading to thyroid follicular neoplasia.

2. Seminiferous tubule destruction by cadmium leading to Leydig-cell tumours of the testis.

3. Enhanced calcium absorption leading to adrenal medullary hyperplasia.

4. Overfeeding leading to nephropathy and via disturbed calcium homeostasis to adrenal medullary neoplasia.

Table 5 Incidences of certain endocrine tumours in male rats exposed to a diet containing 20% lactose (50/group)

	Control	20% lactose	
% of rats with adrenal medullary hyperplasia or neoplasia	36	68	+
% with phaechromocytoma	20	40	+
% with malignant phaechromocytoma	6	18	
% with Leydig-cell tumour	4	24	+
% with pancreatic islet-cell tumour	14	2	-
% with pituitary tumour	24	14	

\+ = significantly higher than expected ($p<0.05$)

\- = significantly lower than expected ($p<0.05$)

Table 6

Effects of Bromocriptine in female rats
(Richardson et al, 1983)

	Dose (mg/kg/day)			
% incidence	0	1.8	9.9	44.5
Moderate/severe nephropathy	16	8	0^{--}	0^{--}
Pituitary tumour	7	8	3	1
Mammary tumour	40	15^{--}	10^{--}	8^{--}
Endometrial inflammation/ hyperplasia/metaplasia	5	35^{+++}	41^{+++}	42^{+++}
Uterine tumour	0	2	8^{++}	9^{++}

++/-- = $p<0.01$
+++/--- = $p<0.001$

Table 7

Effect of overnutrition on survival andrenal disease in rats (Harleman et al, 1984)

	Sex	24 hours access/day	6.5 hours access/day
Survival to 2 years	♂	8/20	18/20
	♀	14/20	16/20
Incidence of moderate	♂	13/20	1/20
or severe nephropathy	♀	12/20	0/20

Table 8

Association between grade of nephropathy and adrenal medullary hyperplasia/neoplasia in 196 male rats aged 26 months

	Nephropathy Grade	
	0-2	3-5
Adrenal medulla		
Hyperplasia grade 0-2	106	48
Hyperplasia grade 3-5 and/or phaeochromocytoma	12	30

$X_2 = 20.8$
$p<0.0001$

Main Conclusion

1. The usual sequence in hormonal carcinogenesis is probably proliferation first and mutation second.

2. Ad libitum-fed (= grossly overfed) rats, with chaotic endocrine disturbances, are wholly unsuitable for the purpose of carcinogenesis bioassay.