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# Adrenal Gland: What do high incidences of hyperplastic and neoplastic changes in long-term rat studies mean?

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The various endocrine glands act as a physiological orchestra under the baton of the pituitary gland. It is therefore unrealistic to attempt to interpret the significance of proliferative changes in the adrenal medulla without at the same time taking into account endocrine status generally. In the time available it is scarcely possible for me to do this even in relation to classical hormones let alone to the rapidly expanding knowledge of peptide hormones. The most I can do is to identify signposts for future research and to set down reasons for doubting the value of an observed increased incidence of medullary neoplasms in laboratory rats is of any use for predicting adrenal or general carcinogenicity in humans.

The adrenal medulla consists essentially of 2 types of cell: chromaffin cells and ganglion cells. The former contain granules which turn brown on exposure to chrome salts. Sub-populations of chromaffin cells produce adrenalin and noradrenalin, respectively. These sub-populations are probably innervated separately. Figure 1 illustrates the types of proliferative and neoplastic change which histopathologists encounter in the adrenal medulla of laboratory rats.

The types of lesion most commonly encountered in the adrenal medulla of rats are hyperplasia, and benign and malignant neoplasms of chromaffin-negative cells. The distinctions between hyperplasia and neoplasia and between benign and malignant neoplasms is often difficult. Hollander and Snell (1976) ducked the problem by deciding to call all proliferative lesions "phaeochromocytomas" However, when an an expert on the human adrenal gland looked at a collection of rat

adrenal glands previously reported by an experienced small animal pathologist as showing hyperplasia or benign neoplasms, he was inclined to regard all the lesions as only hyperplastic (Symington, 1979). In my opinion, despite the undoubted difficulties, it is essential to retain the term hyperplasia for single or multiple foci of proliferating small chromaffin-negative cells and at least try to distinguish between benign and malignant neoplasms according to stated criteria. I apply the term "benign phaeochromocytoma" to discrete, non-invasive expanding lesions which show compression of surrounding tissue, and the term "malignant phaeochromocytoma" to actively invasive expansive lesions and to lesions which exhibit nuclear atypia. Metastasis is too rare to be of practical use as a criterion for malignancy.

Slide 2: Adrenal medullary hyperplasia

Slide 3: Benign phaeochromocytoma

Slide 4: Benign phaeochromocytoma showing absence of chromaffinity.

Cheng (1980) reviewed the incidence, aetiology, morphology and functional activity of phaeochromocytomas in rats. Although the risk of development of such neoplasms is undoubtedly partly genetically determined, a mythology concerning strain differences has built up because investigators have underestimated the contribution of environmental factors and failed to investigate their possible role. From an overview of the available data, three general facts emerge:

1. Within nominally the same rat strain, wide variation occurs in the incidence in untreated animals of proliferative and neoplastic changes in the adrenal medulla.

2. In most strains males are more susceptible than females.

3. The majority of lesions, both hyperplastic and neoplastic, are chromaffin-negative.

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Table 1 illustrates the variation in incidence within nominally the same strain and the fact that males are usually more affected than females.

Catecholamines released from the adrenal medulla are stable in blood owing to the presence of antioxidants, such as ascorbic acid. However, in the tissues both adrenalin and noradrenalin are quickly inactivated. In man inactivation is by methylation to metanephrine and subsequent side-chain oxidation to vanilloyl-mandelic acid (VMA, 3 methoxy-4-vanilloyl mandelic acid). Thus in humans, in whom most phaeochromocytomas are funcitonal, a raised urinary level of VMA is diagnostic for the presence of such tumours. In rats the main urinary metabolite of catecholamines is 3 methoxy-4-hydroxyphenylglycol (MHPG)

Recently, Bosland and Baer (1983) examined 115 aged male Wistar rats for evidence of functioning phaeochromocytoma. At necropsy, 55 of the rats had hyperplastic and 25 had neoplastic lesions in the adrenal medulla. However, during life none of these animals exhibited raised blood pressure, and in none of them were excessive levels of VMA or MHPG found in the urine. Furthermore, most of the lesions were either chromaffin negative or only feebly chromaffin positive. Only 1 out of 25 phaeochromocytomas examined showed marked chromaffinity and this was aged 30 months when killed

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Whilst non-functionality is a feature of virtually all 'spontaneously-arising' phaeochromocytomas in the rat, there are reports in the literature of the apparent induction of chromaffin-positive, functioning tumours by certain agents including thiouracil (Marine and Baumann, 1945; Hopsu, 1960), growth hormone or oestrogen (Lupulescu, 1959, 1960, 1961) and alloxan (Grasso, 1963)

## Environmental factors known to influence the incidences of adrenal medullary hyperplasia and neoplasia

### Reserpine

In the NCI's Bioassay of Reserpine for possible carcinogenicity (NIH, 1979), Fischer 344 rats were fed on diets containing 5 or 10 ppm of the drug for 2 years. As in virtually all long-term experiments in which rats are fed ad libitum a high incidence of pituitary tumorus were seen in both sexes of all groups and high incidences of mammary fibroadenomas were seen in females of all groups. Males

on the higher level of reserpine developed significantly fewer pituitary tumours than the controls, but males of both treated groups exhibited significantly higher incidences of phaeochromocytomas (Table 2)

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The authors of the report concluded "Reserpine was carcinogenic in male rats" and suggested that it might also have been so for females had the doses been higher. They did not question the suitability of a model in which over a third of the controls of both sexes had pituitary tumours. Nor did they think to mention the beneficial effect of treatment on the incidence of these tumours in males.

Reserpine, which of course gives negative results in the Ames test, blocks the ATP-catalysed uptake of noradrenaline by intracellular organelles in which it is normally protected from destruction by cellular monoamine oxidase (Koelle, 1975). Consequently, exposure to reserpine results in catecholamine depletion in tissues. It may well be that such depletion constitutes a stimulus for the proliferation of adrenal medullary cells, particularly in the male rats.

A neuroleptic drug which acts by dopamine-receptor blockade

The purpose of my including these data is not to highlight adverse data in relation to a particular drug but to illustrate 3 interrelated points:-

(i) The incredibly high incidence of tumours of endocrine tissues that may occur in untreated control rats.in all 3 experiments but especially in the first.

(ii) The fact that in apparently similarly designed tests ir nominally the same strains of rats, very different results may be seen in terms of effect on the incidence of phaeochromocytomas.

(iii) The greater the evidence of endocrine abnormality in control rats the more likely treatment is to be associated with effects - adverse or beneficial-on tumour incidence

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Table 3 summarises the endocrine tumour data for control rats in the 3 studies. You will have deduced that most animals of both sexes had endocrine tumours of one or more sites, that the overall incidence was highest in Study 1 and lowest in Study 2 althouth these were both conducted in so-called Wistar strains.

A thoughtful investigator looking at these figures might well wonder whether animals showing these high incidences of major endocrine disturbances are appropriate models for studying the carcinogenicity of a xenobiotic agent. Be that as it may, Table 4 shows the effect of Drug X on the incidences of these tumours in the 3 experiments.

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Thus in one out of 3 studies, exposure to Drug X significantly increased the incidence of phaeochromocytomas in males from a control level of 18% while not doing so in females where the control level was 22%. Although the drug in question is a dopamine blocker and dopamine is the precursor of adrenaline in the adrenal medulla, it is impossible to interpret the observations in view of the multiplicity and severity of the disturbances of endocrine status of the control rats in the study.

Like reserpine, Drug X gave completely negative results for mutagenicity and clastogenicity in a battery of in vitro and in vivo tests.

Various polyols

Data from long-term studies in which rats have been exposed to xylitol, sorbitol or mannitol, although not, I believe, formally published, have been sufficiently widely disseminated for me to say, without revealing any secrets, that all of them are associated with proliferative changes in the adrenal medulla of rats. I have recently seen similar but confidential data for another agent and am satisfied that the effect on the adrenal medulla is a generic one for sugar alcohols in rats.

Table 5 briefly summarises some of the data from one study in which rats were given diets containing 20% xylitol and sorbitol. Non significant increased incidences of adrenal medullary hyperplasia were seen in males fed 5% or 10% xylitol in this study. No comparable data for dietary concentrations of sorbitol less than 20% are available.

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The evidence that mannitol causes adrenal medullary changes is more equivocal Gonwer et al (1978) studied the effects of prolonged exposure to a 10% mannitol diet in female rats of 3 strains: Fischer 344, Sprague-Dawley and Wistar. In the Fischer strain there was a suggestive increase in the incidence of hyperplasia and phaeochromocytoma, but no such effect was evident in the other two strains.

In the case of the sugar alcohols which I am not free to name, 10% incorporation in the diet seemed marginally to increase the incidence of both hyperplasia and phaeochromocytomas.

The different ~~hexitols~~ polyols share certain features in common. They all cause caecal enlargement and, in excessive dosage, diarrhoea, they all cause increased absorption and urinary excretion of calcium and they all predispose to pelvic nephrocalcinosis in rats. All these features they share with the sugar of mother's milk, namely, lactose.

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It occurred to me in the case of the ~~hexitols~~ polyols that the adrenal medullary changes may be secondary to their effects on calcium metabolism, but if so, then one would certainly expect to see adrenal changes in rats exposed to high dietary levels of lactose. My efforts to check this out were at first frustrated because although there had been many studies on lactose, no-one had taken sections of the adrenal glands. However, I soon chanced upon a recent paper by Brion and Dupuis (1980) These investigators reported that the hypofunctioning of the adrenal medulla associated with vitamin D deficiency in rats can be corrected by feeding lactose.

Their data suggested that lactose exhibits this beneficial effect by correcting the hypocalcaemia due to the vitamin deficiency.

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Is it possible that hypercalcaemia resulting from exposure to unphysiologically high levels of lactose in the diet could lead to over-functioning and hyperplasia of the adrenal medulla?

The first evidence that supported this theory came from a small study of our own in which 20 female rate were given a diet containing 30% lactose from the age of 9 months until they were killed at the age of 18 months. Although we had not looked at the adrenals when we reported the study (Hodgkinson et al, 1982) we had preserved them in fixative. In 3 of the 20 lactose-fed rats we found florid adrenal medullary hyperplasia and similar but less marked changes were evident in 2 other animals. By comparison, none of 20 control females showed hyperplastic changes.

More recently, far more convincing data have come into my possession from a study which is otherwise confidential. The lactose and control data from this study conducted in a Wistar-derived strain of rats are summarised in Table 6

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As in the case of the ~~hexitol~~ Polyol-induced lesions, the lactose-induced lesions were almost entirely chromaffin-negative.

These data for lactose and various hexitols do not of course prove that increased calcium absorption is the proximate cause of the proliferative changes in the adrenal medulla associated with the feeding of these agents, but this is arguably the best theory we have at the present time.

It has long been known that calcium is important, not only in the release of acetylcholine from nerve endings, but also in the secretion of catecholamines by chromaffin cells (Houssay and Molinelli, 1928; Douglas and Rubin, 1961; Perlman and Chalfie, 1977). Pawlikowski (1982) proposed that changes in intracellular

hormone content of cells such as those of the adrenal medulla, may serve as a stimulus for mitosis of those cells. He also proposed that external mediators may stimulate both hormone secretion and proliferation, and put calcium ions, cyclic nucleotides and prostaglandins at the top of his list of possible mediators. Clearly, a whole new area for interesting basic research is opening up and the discovery of the effects of lactose on the adrenal medulla offers a new tool for research in this area.

Implications for man of adrenal medullary proliferative changes in the rat

For whatever reason, phaeochromocytoma is rare in man, and there is no human equivalent of the high spontaneous incidences that are commonly seen in laboratory rats of various strains, particularly in males. Furthermore, the phaeochromocytomas of humans are virtually all chromaffin-positive and associated with clinical evidence of excessive catecholamine release whilst most of those in the rat are non-functional. Thus it is, ab initio, difficult to find a human disease for which the chromaffin-negative proliferative lesions of the rat adrenal medulla are a model.

A perusal of the medical literature has failed to reveal any association between hypercalcaemia in humans and disorders of the adrenal medulla. According to Wrong (1982), only one out of 700 cases of primary hyperparathyroidism, all of whom had hypercalcaemia, operated upon at University College Hospital in London had a phaeochromocytoma and this patient may well have been an undianosed case of familial multiple endocrine adenomatosis.

The extensive studies of Fournier and his colleagues in France (Fournier et al, 1971) showed that in rats and mice, lactose and certain monosaccharides such as galactose, given orally enhance the absorption of calcium from the gut. They pointed out that this activity is of physiological importance in rapidly growing mammals generally. However, the evidence for this effect of dietary lactose is far more extensive and persuasive for the rat and the mouse than for man. In the case of the hexitols, it is easy to show that high dietary levels lead to increased urinary calcium excretion in rats and mice, but no evidence of any such effect has been observed in humans.

Although the effects of ~~hexitols~~ polyols on calcium absorption and excretion are similar in rats and mice, the effects on the adrenal medulla are peculiar to the rat which, for some unknown reason, is unduly susceptible to the spontaneous development of adrenal medullary proliferative changes. In this respect man seems to be more like a mouse than a rat.

Clearly, there are a lot of unanswered questions and many new opportunities for investigative research. However, an overview of the available data suggests that proliferative adrenal medullary changes in rats, particularly under conditions of high background incidence in control animals, are not predictive of any risk of adrenal pathology in man.

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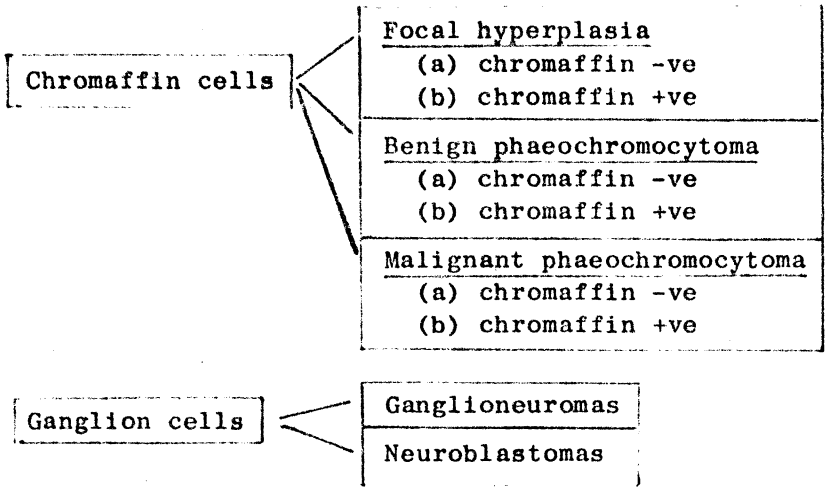
Slide 1

FIGURE 1

**Proliferative and neoplastic lesions of the rat adrenal medulla**




Slide 2 Photomicrograph depicting

Adrenal medullary hyperplasia
- untreated male rat

Slide 3 Photomicrograph depicting

Adrenal medullary hyperplasia
and benign phaeochromocytoma
- untreated male rat

There is no Slide 4

Slide 5

Main features of proliferative and neoplastic lesions of adrenal medulla in rats

1. Wide variation in incidence within same strain
2. Males more susceptible than females
3. Most lesions are chromaffin negative

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Table 1: Reported incidences of phaeochromocytomas in male and female rats of 3 different strains

| Strain | % in untreated | | Reference |
|---|---|---|---|
| | males | females | |
| Wistar-derived | 81 | 56 | Gillman et al, 1953 |
| " | 0 | 2 | Boorman and Hollander, 1972 |
| Sprague-Dawley | 51 | 8 | Kociba et al, 1979 |
| " | 16 | 4 | Thompson and Hunt, 1963 |
| Fischer 344 | 37 | 12 | Jacobs and Huseby, 1967 |
| " | 4 | 0.5 | Sass et al, 1975 |

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Agents which produce functional (chromaffin-positive phaeochromocytomas

Rats and mice

Thiouracil - Marine and Baumann, 1945
- Hopsu, 1960

Rats and guinea pigs

Growth hormone* Lupulescu, 1959, 1960,
Oestrogen 1961

Rats

Alloxan - Grasso, 1963

* but Moon et al (1950) produced chromaffin--ve adrenal medullary tumours in rats with growth hormone

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Table 2: NCI Study on Reserpine in groups of 50 F344 rats

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| | Control | Low dose | High dose | Control | Low dose | High dose |
| Phaeochromocytoma | 3 | 18*** | 24*** | 1 | 3 | 4 |
| Pituitary adenoma | 17 | 13 | 6** | 21 | 27 | 28 |
| Mammary fibro-adenoma | | | | 14 | 18 | 14 |

*** $p < 0.001$

** $p < 0.01$

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Table 3: Endocrine tumours in control groups (%) in 3 separate 2 year carcinogenicity studies on Drug X

| Study No. | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Strains of rat | Wistar | | Wistar | | Sprague-Dawley | |
| Sex | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Pituitary | 22 | 62 | 17 | 53 | 41 | 46 |
| Benign mammary | 0 | 86 | 2 | 11 | 8 | 77 |
| Malignant mammary | 0 | 10 | 0 | 0 | 2 | 22 |
| Phaeochromocytoma | 18 | 22 | 0 | 0 | 2 | 0 |
| Adrenal cortex | 10 | 10 | 0 | 0 | 0 | 3 |
| Thymoma (endocrine type) | 4 | 10 | 0 | 0 | 0 | 0 |
| Thyroid - follicular | 26 | 18 | 0 | 0 | 0 | 0 |
| - C-cell | 0 | 6 | 9 | 5 | 1 | 0 |
| Pancreas - islet cell | 4 | 0 | 4 | 3 | 4 | 5 |
| Parathyroid hyperplasia | 25 | 7 | 0 | 0 | 0 | 0 |

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Table 4: Effect of Drug X on endocrine tumour incidence in 3 long-term rat studies

| Study | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| Strain | Wistar | | Wistar | | Sprague-Dawley | |
| Sex | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ |
| Pituitary | ↑ | 0 | 0 | 0 | 0 | 0 |
| Mammary | ↑ | ↑ | 0 | 0 | ↑ | 0 |
| Phaeochromocytoma | ↑ | 0 | 0 | 0 | 0 | 0 |
| Thymoma (endocrine) | ↑ | ↑ | 0 | 0 | 0 | 0 |
| Thyroid - follicular | ↓ | 0 | 0 | 0 | 0 | 0 |
| Pancreas - islet cell | ↑ | ↑ | ↑ | ↑ | 0 | 0 |

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Table 5: % rats with adrenal changes after exposure to xylitol or sorbitol for 79 or more weeks

| Males | Control | 20% Xylitol | 20% Sorbitol | 20% Sucrose |
|---|---|---|---|---|
| Medullary hyperplasia | 10 | 29 | 38** | 6 |
| Phaeochromocytoma | 17 | 31 | 13 | 6 |
| Females | | | | |
| Medullary hyperplasia | 5 | 25** | 17 | 3 |
| Phaeochromocytoma | 2 | 14 | 11 | 8 |

** $p < 0.01$

Russfield (1981) re-evaluation of Hunter et al (1978)

Slide 12

Similar responses of rats to polyols and to lactose

Caecal enlargement

Increased absorption of calcium from the gut

Increased urinary excretion of calcium.

Slide 13

Brion and Dupuis Canad. J. Physiol. Pharmacol., 58, 1431, 1980

1. Correction of hypocalcaemia in vitamin D-deficient rats restores adrenal medullary hypofunction to normal.

2. Hypocalcaemia corrected by 20% lactose in diet.

Slide 14

Table 6: Effect of the long-term feeding of a diet containing 20% lactose on the adrenal medulla of rats

| | Control | | 20% lactose | |
|---|---|---|---|---|
| % rats with:- | ♂ | ♀ | ♂ | ♀ |
| Malignant phaeochromocytoma | 7 | 0 | 20 | 2 |
| Benign or malignant phaeochromocytoma | 23 | 2 | 44 | 4 |
| Hyperplasia or phaeochromocytoma | 41 | 16 | 71 | 26 |
| Bilateral proliferative medullary changes | 18 | - | 29 | - |