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# The body has a brake: micrin is a postulated new gonadal hormone curbing tissue overgrowth and restricting reproduction

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ABSTRACT

There is evidence for an unrecognised classical hormone secreted by the mammalian gonad. This postulated hormone – 'micrin' (pronounced 'my-crin') – represents the body's brake against tissue overgrowth. When oestrogens are administered in high doses to female rats there is a considerable (non-artefactual) increase in the relative size and weight of organs such as the pituitary, adrenals, uterus and liver – suggesting an organotrophic (organ-building) role for endogenous oestrogens. This effect is exaggerated if the animals are first ovariectomized, indicating the removal of a negative ovarian factor, micrin. These organ enlargements can be reduced by pretreating the rats with large doses of antioestrogens such as clomiphene and tamoxifen. This antiestrogenic blockade of exogenous oestrogens is itself blunted by prior removal of the ovaries. It is proposed that antioestrogens (e.g. tamoxifen in breast cancer treatment) antagonize the organotrophic effects of oestrogens by competing for the oestrogen receptor peripherally and centrally and via an increase in the secretion of ovarian micrin. It is deduced that micrin is the testicular 'inhibin' proposed in the 1930s, not the molecule that now bears that name, which acts at the pituitary tier as a downregulator of follicle-stimulating hormone. The hallmark of micrin deficiency in the male rat is a pituitary hypertrophy that follows castration. This is reversible with a steroid-depleted aqueous bovine testicular extract, the micrin within which suppresses the hypothalamus, normalizing the pituitary. Micrin probably acts as a brake on peripheral tissues directly but also indirectly at the meta-level via the hypothalamic–pituitary axis, resetting a hypothalamic 'organostat' controlling organ and tissue masses, part of the 'organotrophic system' of internal size regulation. Besides endocrine (circulating) micrin from the gonads there is probably paracrine (locally acting) micrin produced in the brain. This is involved in a somatic cueing system for puberty: the brake comes off at an appropriate body tissue mass disinhibiting the hypothalamus and accelerating the organism towards sexual maturity and full adult stature. This suggests the use in reproductive disorders of micrin-related drugs. These could also be inhibitors of breast, prostate and other cancers, while protecting the bone marrow via a trophic effect on the adrenals (the lack of which protection causes lethal bone marrow depression in oestrogen-treated ferrets and dogs). Benign prostatic hyperplasia is asserted to be a micrin deficiency disorder, involving insufficiently opposed androgen. The rise in cancers with age could be associated with a reduction in micrin protection and a relative lack of this hormone could partly explain why men die younger than women. Micrin is dissimilar in activity to any known molecule and could usefully be isolated, characterised and exploited therapeutically.



## Introduction

The totality of endocrine, paracrine, autocrine, neurological and other influences on the masses of bodily tissues and organs has been described as the 'organotrophic system' [1]. A new negative, downregulatory, endocrine component of the system is here postulated, 'micrin' (Greek, 'small', as in small organs). Evidence for this new humoral entity comes from studies in rats and ferrets involving removal of endocrine glands and from experiments in these and other animals involving the administration of oestrogens and antioestrogens in high doses (Table 1 and Fig. 1). The micrin hormone hypothesis is freighted with human therapeutic implications (section "Therapeutic opportunities").

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## Hypothesis formation

### *Rat*

Oestrogens are the most organotrophic substances known [1]. In rodents, carnivores and ruminants they have been shown to

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induce widespread gains in organ masses. Other compounds which display organotrophic effects often do so only in regard to a single organ, usually the liver [2] or the adrenals [3]. In one experiment the synthetic nonsteroidal oestrogen hexoestrol, a bibenzyl derivative, was given orally at the ultra-high dose of 60 mg/kg of body weight to mature ovary-intact female rats [4]. The relative organ weights were calculated as the ratio of the organ to the body expressed as a percentage, from absolute organ weights and body-weights in grams. The following changes were provoked in the relative organ weights after 4 days dosing: pituitary (+23%), adrenals (+43%), ovaries (+13%), uterus (+49), liver (+37%), kidneys (+3%) and spleen (−11%). The heart weights were unchanged. These changes are due to cellular hyperplasia (increase in number) and hypertrophy (increase in size), except for the decline in spleen weight, the morphological character of which has not been established [1]. They are not artefacts of alterations in body weight and the changes are sustained or increased with continuing oestrogen treatment [5]. To virtually eliminate interpretative difficulties caused by body weight variations [6] studies of a few days duration can be performed using rats whose age is such that their velocity of growth compensates for the appetite- and growth-suppressing effects of oestrogens and antioestrogens [1]. The visceral organs under review, together with the pituitary, comprise about 5% of total body mass. Quite large reductions can occur, as here, without registering significantly on body weight.

Little has been made of the organotrophic properties of oestrogens. It would seem that these have been undervalued as non-specific 'toxic' effects, in spite of the paucity of histopathological evidence of toxicity. It is worthwhile in a heuristic sense to adopt a different viewpoint, by treating these organ weight changes as exaggerated endocrinology [1]. From this flows a perception: in the untreated rat, physiological oestrogens exert an upregulatory influence on soft tissue masses (Table 1, Item 1 et seq).

In the oestrogen experiment just described [4], the organotrophic effects were also determined of clomiphene citrate, an antioestrogen used clinically to induce ovulation [7,8]. Clomiphene is related to tamoxifen, which is itself widely used in the prevention and treatment of breast cancer. Both are triphenylethylene derivatives and both are regarded as antioestrogens, though they are more properly described as mixed agonist/antagonist oestrogen receptor ligands, within the class of selective oestrogen receptor modulators [7,8]. The clomiphene dose level of 60 mg/kg/day is an order of magnitude above the highest doses employed in humans. The following changes in the relative organ weights were seen in adult female rats after 4 days dosing with clomiphene alone, without there being body weight changes and with no overt toxicity: pituitary (−13%), adrenals (+7%), ovaries (−4%), uterus (−25%), liver (−18%), kidneys (−11%), spleen (−17%), and heart (−16%). In a further group of rats clomiphene at pretreatment doses of 20, 40 and 60 mg/kg/day eliminated in a dose-dependent fashion the organ enlargements caused by hexoestrol administered at 60 mg/kg/day. The exception was the adrenals, whose hexoestrol-induced weight gain was exaggerated by clomiphene pretreatment – an adrenotrophic effect that endures with continuing clomiphene and oestrogen treatment [5]. Tamoxifen stunts a rise in pituitary weight which is otherwise provoked in the rat by exogenous oestradiol [9].

An obvious explanation for clomiphene's antiorganotrophic action when administered alone would appear to be that it is antagonizing endogenous oestrogens. Clomiphene is known to compete for the high affinity intracellular receptors, both $\alpha$ and $\beta$ subtypes, through which oestrogens act in peripheral tissues and centrally at the hypothalamus and pituitary [7,8]. In regard to a central site of action, a meta-level locus would account economically for the wide-ranging antiorganotrophic effects of clomiphene on its own and for the similar dose-dependent pattern of stepwise decrements in weights seen across the organs of rats treated with hexoestrol and rising doses of clomiphene. In this picture clomiphene treatment is akin to chemical ovariectomy. In mice, clomiphene administered at 0.5 mg/kg/day for 7 days reduces the weight of the liver, but only in the presence of intact ovaries [10]. Ovariectomy involves the removal of the main source of the endogenous oestrogens – notably oestradiol – causing uterine atrophy. In one study serum oestradiol was not detectable in rats 4 weeks after ovariectomy [11], though total oestrogen will not have been zero, as there are other minor sources of less potent versions of this hormone besides the ovaries (e.g. adrenals, fat cells). So does ovariectomy itself lead to organ reductions, uterine shrinkage aside? Yes, but the effects are less marked than those elicited by clomiphene, slower to assert themselves and the effects are in any case partly an artefact of increased body weight [1]. Even granted that endogenous oestrogens have a larger (tonic) role than has hitherto been recognised in the organotrophic system, clomiphene in terms of antiorganotrophic effects is anomalously potent.

Clomiphene like other weak agonists displays oestrogenic activity when endogenous oestrogens are low, but is antioestrogenic when levels are normal, as in the present context [12]. Clomiphene is regarded as an agonist at the liver [7, p. 134]. If clomiphene were acting here like a weak agonist or a mixed agonist/antagonist we might expect to see some (oestrogenic) organotrophic changes (e.g. a boosted liver weight), but we do not (though maintenance of adrenal mass is unexpected, given pituitary regression; see below). So the comparison between clomiphene treatment and ovariectomy seems reasonable, since there would appear to be a paucity of agonist-like effects requiring explanation.

Clomiphene on its own significantly reduces the spleen and heart weights in the rat but not that of the adrenals. This suggests a supportive organotrophic role for endogenous oestrogens in the cases of the spleen and heart at least. In turn this predicts that exogenous oestrogens will boost the splenic and cardiac masses while having little effect on the adrenals, yet exogenous oestrogens administered alone are antiorganotrophic as regards the spleen, neutral as regards the heart and profoundly adrenotrophic [4]. So clomiphene is not merely antiorganotrophic to an anomalous degree but is so in a pattern that is not adequately connoted by the words 'antioestrogen' or 'oestrogen'.

What of the functional and structural status of these expanded and shrunken organs? Hepatomegaly induced by hexoestrol is associated with a decrease in vitamin K-dependent blood clotting in the rat, secondary to a reduction in the liver synthesis of clotting factors [13]. Thus is enlargement associated with hypofunctionality, as in the iodine-deficient goitrous thyroid. Clomiphene administered daily at 0.5 mg/kg for 7 days significantly decreased the weight of the mouse liver and its RNA and protein content, while causing a downward trend in cell numbers and DNA [10]. An organ by organ analysis could in fact be attempted to establish functional and structural changes [1], but the 'escape' thought is to privilege organ size (volumetric magnitude) itself and focus on endocrinology.

Hypophysectomy (pituitary removal) reduces or eliminates the oestrogen-induced organ weight changes seen in the mature ovary-intact female rat [4]. This indicates the presence of a mediating or co-operative pituitary influence in the organotrophic effects of oestrogens. In the case of the uterus, a direct uterotrophic effect of oestrogens is of course well established. For full uterine growth, though, pituitary hormones seem to be required and the secretion of these is presumably influenced directly by oestrogens acting centrally. The pituitary hormones of relevance here are GH and thyroid-stimulating hormone (TSH), the latter increasing thyroxine [1].

GH is also implicated in oestrogen-induced hepatomegaly [4]. As with the uterus, a direct oestrogen effect can be posited,

**Table 1**
Observations and deductions from studies in the rat and ferret.

| Item | Observations | Deductions |
|---|---|---|
| 1 | Rat: exogenous oestrogens are organotrophic in the female rat [4,5]. They provoke non-artefactual gains in the relative organ weights of the pituitary, adrenals, ovaries, uterus, liver and kidneys, due to a mixture of cellular hypertrophy and hyperplasia [1] | Endogenous oestrogens exert a mostly upregulatory (tonic) influence on soft tissue masses in the female rat |
| 2 | Oestrogen-induced organomegaly in the rat, e.g. involving the uterus and liver, involves a direct oestrogenic trophic effect and an indirect pituitary contribution, via e.g. GH [4,1] | Oestrogens, exogenous and endogenous, act locally and centrally to organotrophic effect |
| 3 | The organotrophic effects of high doses of exogenous oestrogens in the female rat can be blocked in a dose-dependent manner by high-dose clomiphene antiestrogen pretreatment [4] | Oestrogens are broadly organotrophic and antioestrogens broadly antiorganotrophic at high and low doses |
| 4 | Clomiphene, when administered alone in high doses to ovary-intact female rats, has profound tissue-shrivelling properties, with a number of organs (liver, spleen, heart, kidneys, pituitary, uterus) reduced in size to below control values [4] | Given that this effect presumably includes antagonism of endogenous oestrogens, the notion is supported that endogenous oestrogens are broadly organotrophic; yet the antiorganotrophic effects of clomiphene are surprisingly more marked and consistent across multiple organs than might be expected from antagonism of endogenous oestrogens alone; an additional effect is involved |
| 5 | Selected organs from Item 4: clomiphene, when administered alone in high doses to ovary-intact female rats, reduces rat spleen and heart sizes, while leaving the adrenals unchanged [4] | On the simple blockade theory of antioestrogen action, these clomiphene effects predict that oestrogens should be splenotrophic, cardiotrophic and adreno-neutral. In fact exogenous oestrogens in the rat are anti-splenotrophic, cardio-neutral and adrenotrophic. Clomiphene's antiorganotrophic activity is not based solely on antagonism of oestrogen |
| 6 | Ovariectomy in the untreated rat is antiorganotrophic, but much less so than the 'chemical ovariectomy' of clomiphene-only treatment [1,4] | Ovariectomy is not simply the loss of a positive (oestrogenic) influence on tissue masses, but also the loss of a negative ovarian influence, micrin, hence it is milder than would be predicted on the basis of removal of organotrophic endogenous oestrogen alone; and clomiphene treatment does not only involve blockade of endogenous oestrogens locally and centrally, but the induction of micrin, so its antiorganotrophic effects are more marked than would be predicted from oestrogen antagonism alone |
| 7 | Ovariectomy in the rat boosts the pituitary [16,17], adrenal [17,18], uterine [17,19] and hepatic [20] weight gains induced by exogenous oestrogens | As well as the positive ovarian influence on soft tissue masses represented by endogenous oestrogen, there is a negative ovarian influence, micrin |
| 8 | Ovariectomy suppresses the antiorganotrophic effects of clomiphene and those of the related antioestrogen tamoxifen in the oestrogen-treated rat [9 v 4,19] | The organ-shrinking effects of these compounds cannot be explained solely on the basis of antagonism of exogenous oestrogen (still less on the basis of partial agonism, which would predict organ enlargements, rather than the reverse); there would appear to be an antiorganotrophic effect mediated via ovarian micrin |
| 9 | Male rat: castration causes pituitary hypertrophy [22]. This effect is associated with the appearance of 'castration cells' [23], indicating the existence in the intact animal of an inhibitory gonadal influence on the hypothalamic-pituitary unit. The pituitary hypertrophy and the associated cellular changes cannot be reversed by testicular steroids but can be reversed by the administration of an aqueous testicular extract subject to steroid depletion (notably of testosterone) [25,45] | Using Ockham's razor, the inhibitory testicular influence on the male pituitary is the same as the inhibitory ovarian influence on the female pituitary, micrin |
| 10 | A steroid-depleted aqueous beef testicular extract administered by feeding or injection to female rats disturbs the oestrous cycle [53] | The male inhibitory gonadal factor works in females |
| 11 | The hallmark pituitary hypertrophy of castration in the rat is evoked much more quickly in immature castrates than mature ones [22,27] | The net inhibitory gonadal influence on the pituitary is stronger before sexual maturation than after, consistent with a lifting of the micrin brake at puberty |
| 12 | If a castrated rat and a normal rat are conjoined in parabiosis, then prostatic enlargement results in the normal animal [53]. Treating the castrate with a steroid-depleted aqueous beef testicular extract eliminates the prostatic hypertrophy that would otherwise be seen in the normal animal [53] | Lack of testosterone in the castrate induces pituitary hyperactivity in terms of gonadotropin production, which goads the normal's testes to produce extra testosterone, stimulating growth of the normal's prostate. The male inhibitory gonadal factor stills the castrate's pituitary, preventing enlargement of the normal's prostate |
| 13 | An organotrophic picture of 'pituitary down, adrenals up' is seen in (a) intact male rats given a steroid-depleted aqueous beef testicular extract (where 'prostate down' is also seen)[29]; (b) clomiphene-treated female rats [4]; and (c) clomiphene-pretreated oestrogen-treated female rats [4] | This pattern is attributable to micrin, which though antihypophysiotrophic and generally antiorganotrophic, is yet also adrenotrophic; 'pituitary down, adrenals up' is a micrin signature |
| 14 | A mush of whole bulls' testes causes hypertrophy of the rat prostate when injected, but prostatic atrophy by ingestion [58,59] | Besides testosterone, which is bioavailable by injection but less so orally, there is an orally active testicular antiprostatic factor, micrin |
| 15 | Pituitary hypertrophy, as is seen after castration in the rat, can also be induced by irradiating the testes with X-rays and by transplanting them elsewhere in the animal. These procedures cause tubular degeneration without impairing the interstitial cells [27,66]. The prostate remains normal. If a rat with irradiated testes is linked parabiotically to a normal rat, the latter's prostate undergoes hypertrophy [27] | The interstitial cells produce testosterone, which is why the prostate is maintained. Micrin is evidentally produced in the germinal epithelium of the tubules, accounting for the pituitary hypertrophy, as micrin's inhibitory influence is lost. The prostatic hypertrophy in parabiosis is due to loss of micrin inhibition in the irradiated animal leading, via its own pituitary hyperactivity, to increased testosterone production by the normal animal |
| 16 | Ferret: exogenous oestrogens are organotrophic in the female ferret as they are in the female rat, with increases in size and weight observed for the pituitary, adrenals, ovaries, uterus, liver, kidneys and heart [1,33] | This suggests a similar tonic role for endogenous oestrogens in the ferret as is proposed in the rat |
| 17 | In the ferret ovariectomy does not exaggerate the growth response of the uterus to oestrogen, as it does in the rat [35] | Micrin is not present in the ferret or, more likely, fits into the ferret's endocrinology in a different manner than in the rat; there is a different foot on the micrin brake pedal |
| 18 | Correlating with Item 17 is the observation that clomiphene is neither antiuterotrophic nor generally antiorganotrophic in the oestrogen-treated ferret [33] and administered alone acts as an oestrogen rather than an antioestrogen [36] | Clomiphene, which is antiorganotrophic in the rat partly due to micrin induction, does not induce ferret micrin or, less likely, micrin is lacking in this species |

(*continued on next page*)

**Table 1** (*continued*)

| Item | Observations | Deductions |
|---|---|---|
| 19 | Oestrogens cause lethal bone marrow depression in the ferret, also the dog [1,39]. Clomiphene does not augment adrenal weights in oestrogen-treated ferrets [33] as it does in oestrogen-treated rats [4] | Oestrogen-induced bone marrow aplasia in the ferret and dog is probably due to a runaway pituitary-adrenal derangement. There is an absence in the ferret of the adrenotrophic effect of micrin, which is adaptive in the rat and marrow-sparing |
| 20 | Human: the lethal bone marrow response of the ferret to exogenous oestrogens is not seen in the rat or, mercifully, the human [1]. In the ferret clomiphene does not seem to 'work' antioestrogenically [33], as it does in the rat [4] and human [7,8] | The apparent comparability of the rat and human is important as in the rat clomiphene is the most antiorganotrophic substance known. It has been postulated here that a key element in this clomiphene effect is stimulation of the hypothetical inhibitory ovarian hormone micrin. It is predicted that micrin will prove to be a potent downregulator of soft tissue masses in the human organotrophic system, while being protective of the bone marrow via adrenotrophic adaptation |

together with an essential pituitary co-operation, itself oestrogen-influenced. Oestrogens are known to increase basal secretion of GH, while GH is an important influence on the liver's content of oestrogen receptors [1]. An obvious candidate for the pituitary influence on the oestrogen-elicited adrenal weight gain in the rat is adrenocorticotrophic hormone (ACTH). As for the pituitary weight gain itself, direct action on the adenohypophysis has been postulated [1].

In rats which have been ovariectomized and then treated with oestradiol benzoate in low doses, tamoxifen does not achieve total antagonism of a pituitary weight gain, even when the antioestrogen is administered in massive excess [9]. This is in contrast to the full antagonism of pituitary and other organ weight gains seen in another study involving intact female rats treated with higher, but equal, doses of hexoestrol and clomiphene [4]. This latter result runs counter to a claim that full antagonism cannot be achieved with clomiphene and tamoxifen because they display partial agonism [14]. Total blockade of a uterine weight gain has also been reported in mice given oestradiol benzoate and a much higher dose of HER-25, another antioestrogen with partial agonist activity [15]. Full antagonism seems achievable if the ratio of antioestrogen to oestrogen is colossally in favour of the former or at lower ratios if mature ovary-intact rats are used.

Greater weight increases are seen after exposure to oestrogens in the pituitaries [16,17], adrenals [17,18], uteri [17,19] and livers [20] of ovariectomized rats than of intact animals. It might be expected that exogenous oestrogen plus ovarian oestrogen would sustain *greater* organ masses than exogenous oestrogen without ovarian oestrogens, but it should not be forgotten that exogenous oestrogen will suppress endogenous oestrogen, via negative feedback at the hypothalamic–pituitary axis. So a reasonable expectation would be that exogenous oestrogens should be similarly organotrophic in ovariectomized and ovary-intact rats. That they are markedly more organotrophic in the former than the latter is therefore surprising. The presence of a negative ovarian influence is indicated, which is here being called micrin.

The proposal is that clomiphene and tamoxifen are antiorganotrophic for three reasons: (i) through local antagonism of oestrogens, (ii) central antagonism of oestrogens and (iii) by virtue of an increase in the hypothetical ovarian micrin. In this view, ovariectomy represents the elimination of both a positive (oestrogenic) effect on the pituitary, adrenals, uterus, liver and other organs, and of a negative one (micrin). Clomiphene treatment, meanwhile, would involve a blocked positive ovarian oestrogenic effect and an enhanced negative one, yielding a quantitatively and qualitatively different picture to ovariectomy. In line with this, antioestrogens have been shown to be less antiuterotrophic in oestrogen-treated rats if the animals are first ovariectomized [19].

Ovariectomy in the rat causes pituitary hypertrophy and adrenal hypertrophy in the short term [17,18,21]. The pituitary enlargement is believed to be due to the release of the hypothalamus from the negative feedback influence of oestrogen, leading to hyperactivity of a pituitary keen to stir up the ovary to produce more oestrogen. Hypertrophy is succeeded by hypotrophy, indicating that there is a net positive gonadal influence on pituitary mass in the female rat. Exogenous oestrogens restore pituitary size after ovariectomy, implying a hypophysiotrophic (pituitary boosting) role for endogenous oestrogen. This latter deduction is held to account for the greater size of the female pituitary over the male.

Gonadectomy in the male rat causes an expansion of the pituitary (and of the adrenals) as in the female, but in this case the hypertrophy is enduring, with a doubling reported after an average of 85 days post-castration [22]. A net negative gonadal influence is indicated in the intact male, in contrast to the net positive gonadal influence seen in the female. The pituitary enlargement in the male is due to the appearance of so-called 'castration cells', highly vacuolated signet-shaped basophil cells in the adenohypophysis [23]. Exogenous androgens have little effect on pituitary size, so the elimination of gonadal androgens by castration is not akin to removal of a major influence on pituitary mass. There is a negative feedback effect of testosterone on the hypothalamus, of course, and it is the lifting of this which is usually cited as leading to pituitary hypersecretion and hence hypertrophy. The enlargement is certainly associated in its early stages at least with a rise in the secretion of hypothalamic gonadotropin releasing hormone, GnRH [24]. But the pituitary enlargement of castration is not reversed by administration of either benzene-extracted testicular steroids [25] or testosterone itself [26]. It is eliminated, however, by a steroid-depleted aqueous beef testicular extract [25], which also eliminates the cellular changes [27]. Quote: 'The apparent and only obvious conclusion is that the testicle secretes a hitherto unrecognized water soluble hormone, one function of which is a control of the pituitary gland.' ((McCullagh (1932), 'Dual Endocrine Activity of the Testes') [25]. Seen through the present paper's lens of 'size', rather than function or morphology, what is being described here is a testicular antihypophysiotrophic factor. The use of Ockham's razor dictates that the sought-for antihypophysiotrophic factor in the male is the same as the antihypophysiotrophic (and generally antiorganotrophic) factor identified in the female: micrin.

The adrenal enlargement of castration in the rat, unlike the pituitary enlargement, is counteracted by testosterone [22]. This questions the easy assumption that short-term pituitary and adrenal enlargements seen after ovariectomy are conjoint phenomena. The finding suggests that the post-castration enlargement of the adrenals is due to their release from an antiadrenotrophic effect of testosterone. Experiments indicate that testosterone inhibits the hypothalamic–pituitary–adrenal axis via an effect on the medial preoptic area of the hypothalamus [28]. But that is not the full story. A steroid-depleted aqueous extract of bulls' testes causes the adrenals of the intact rat to

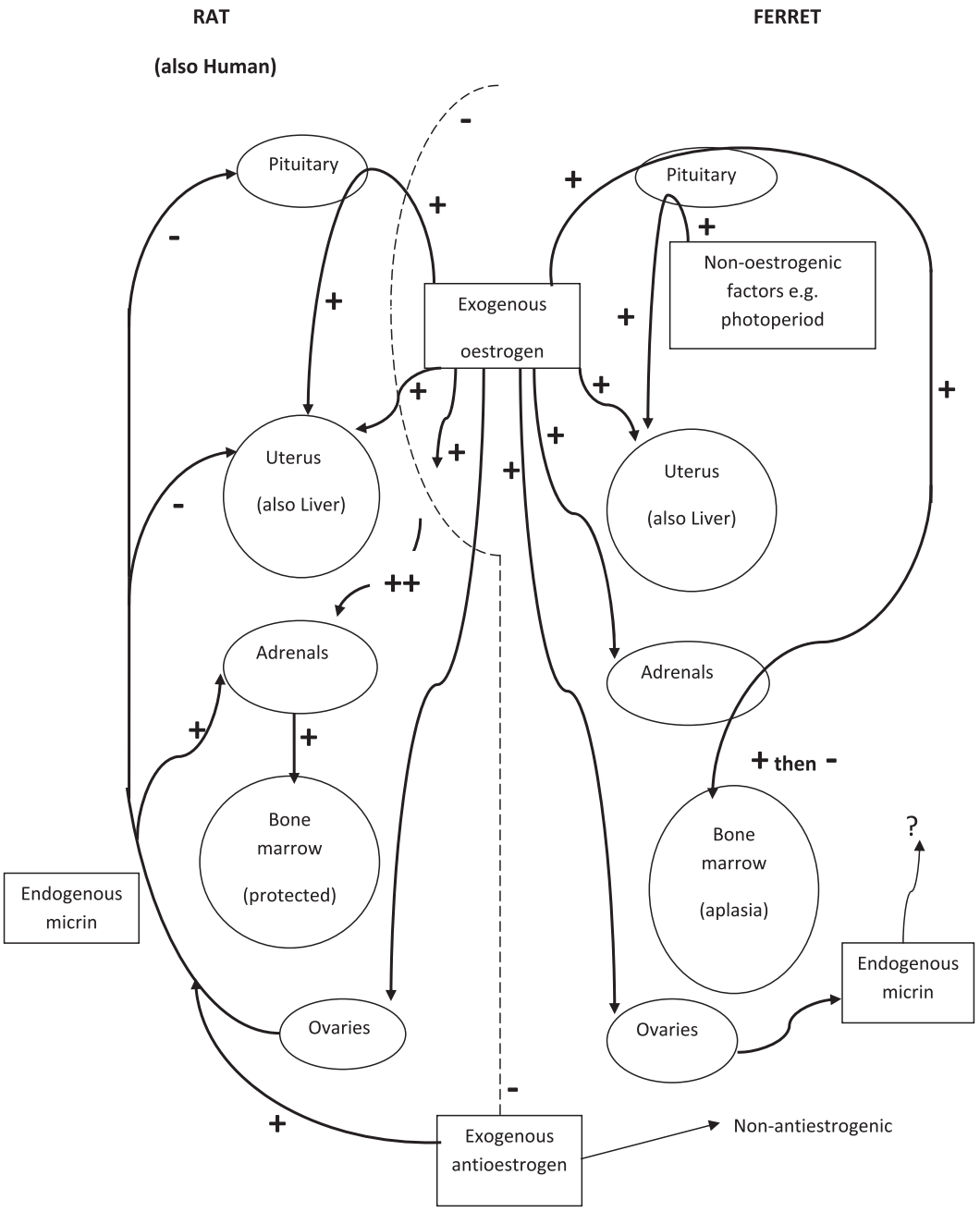


**Fig. 1.** 'Organotrophic map' of organ size influences in female rats and ferrets treated with oestrogen and antioestrogen (clomiphene), showing selected organs in circles, factors in boxes. Arrows denote size influence, with polarity indicated (+ or −). The vertical dotted line denotes antioestrogenic blockade of organotrophic exogenous oestrogen (the adrenals being an exception, with an adrenotrophic effect of exogenous oestrogen exaggerated not blocked in the rat by clomiphene). Endogenous oestrogen effects not shown. Influences omitted for graphical clarity include pituitary trophic effects on adrenals and bone marrow and direct exogenous oestrogen effects on the bone marrow.

expand and the pituitary to shrink [29]. So this is a non-steroidal adrenotrophic effect of the testes as well. Removing this through castration should cause the adrenals to shrink. That they expand tells us that testosterone's antiadrenotrophic effect outweighs the nonsteroidal adrenotrophic effect.

The opposite situation obtains for the post-castration pituitary: as testosterone is not significantly hypophysiotrophic [30] a non-steroidal antihypophysiotrophic effect supervenes, such that post-castration pituitary swells.

The surprising pattern of 'pituitary down, adrenals up' in male rats given an aqueous beef testicular extract is echoed in female rats treated with clomiphene, where 'pituitary down, adrenals maintained or up' is seen [4]. In the same pattern, clomiphene blocks a pituitary rise that would otherwise be evoked by oestrogen while exaggerating an adrenal rise [4]. If clomiphene were acting straightforwardly as an antioestrogen or oestrogen the expectation for the two tissues would be change in the same direction. Clomiphene is believed not to influence ACTH secretion [31], while stimulating adrenal androgen production in the human male [32]. The clomiphene adrenotrophic effect and the equally mysterious testicular adrenotrophic effect can be asserted to have one and the same cause: gonadal micrin. Micrin, though generally antiorganotrophic, is nonetheless disclosed as being adrenotrophic.

*Ferret*

The ferret (*Mustela putorius furo*) is a monoestrous carnivore, a seasonal breeder and an induced ovulator; the rat is a polyoestrous rodent and a year-round spontaneous ovulator. There are similarities and intriguing differences in the response of the two animals to oestrogens and antioestrogens (Fig. 1). Exogenous oestrogens are broadly organotrophic in the female ferret, as they are in the female rat [1,33] (Table 1, Item 16 et seq). This suggests that *endogenous* oestrogens exert a (mainly) tonic effect on the soft tissue masses of the female ferret, as appears to be the case in the female rat. The ferret's response is more individualistic, however, than that of the rat. Oestrogens are unpredictably uterotrophic in the ferret, particularly in the anoestrous animal [33]. In fact, hepatomegaly is more easily evoked than uteromegaly. This doubtless reflects the influence of progesterone and seasonal photoperiodic factors on uterine growth in this species. If instigated, however, the oestrogen-induced uterine growth process in the ferret is more marked than in the rat. This generalisation, and that relating to seasonal susceptibility, is true too of the other organ weight changes, not least to a profound reduction in spleen weight.

The seasonal effect probably relates to the level of pituitary 'activation'. Hypophysectomy reduces uterine and ovarian weights in the ferret [34], as it does in the rat [4]. Interestingly, however, this surgical procedure does not seem to affect the ferret's uterine growth response to exogenous oestrogens [34], in contrast to the rat. This seems to indicate that the pituitary contribution to uterine growth in this day-length sensitive seasonal breeder is not primarily influencable by endogenous oestrogens.

The uterotrophic response of the oestrogen-treated anoestrous ferret is not exaggerated by ovariectomy [35], as it is in the rat. This implies that in the ferret the negative ovarian influence detectable in the rat, micrin, is uninvolved in the ovarian-uterine axis or lacking altogether. The non-operation of a micrin brake might explain the observation that oestrogen-induced uterine weight gains in the ferret, when these occur, are far more dramatic than in the rat and can involve spectacular fluid accumulation [33]. The other oestrogen-induced organ weight changes are more marked too, inviting the speculation that ovariectomy in the ferret will have no more of an augmentative effect on these than it does on that affecting the uterus.

Clomiphene in the ferret is uterotrophic rather than antiuterotrophic as it is in the rat and inhibits pituitary gonadotropin production in the manner of an oestrogen [36] rather than causing a gonadotrophic surge in the style of a centrally blockading antioestrogen. Furthermore, clomiphene is not anti-uterotrophic in the oestrogen-treated ferret either or generally antiorganotrophic or specifically adrenotrophic [33], as it is in the rat. In the same vein, tamoxifen is an oestrogen agonist in the dog and not an antagonist [37]. MER-25 and other compounds which antagonize the uterotrophic activity of oestradiol in rats generally do not do so in hamsters, for unexplained reasons [38]. These creatures all boast oestrogen receptors. Since clomiphene does not appear to blockade oestrogens in ferrets, it can be deduced that clomiphene is not antiorganotrophic in oestrogen-treated ferrets because of the different deployment or non-deployment of micrin in this species.

The bone marrow of the ferret displays a remarkably antiorganotrophic response to oestrogens, to the extent that after a phase of myeloid hyperplasia, reflected in the peripheral blood by leucocytosis, aplastic anaemia is a common cause of death [1,39]. These effects occur in ovariectomized ferrets and are not counteracted by clomiphene. The haematopoiesis toxicity of oestrogens is also seen in extreme form in dogs, but it is not seen in a third species of carnivore, the cat, or for that matter in the rat or human. The first two animals are monoestrous, while the rest are polyoestrous. The bone marrow toxicity of oestrogens may be due to progressive changes affecting the pituitary–adrenal axis [40], according to circumstantial evidence from experiments in the dog [41]. Also suggesting a pituitary involvement is a seasonal susceptibility difference in the ferret, paralleling that for the organ weights, with oestrous animals more prone to respond to oestrogens than those in anoestrus [33]. Among the latter, there is a tendency for degree of haematopoietic toxicity to correlate with intensity of reproductive stimulation, suggesting a set of phenomena which relates to disturbances at a single central locus, the pituitary.

It can be inferred that ferret's bone marrow sensitivity to oestrogens is because micrin is enigmatically inapparent. The particular deficit is the micrin adrenotrophic effect seen in the rat, which is thus revealed to be adaptive and marrow-sparing. The different deployment or absence of micrin is why clomiphene, micrin inducing in the rat, cannot save the oestrogen-treated ferret. In the mouse, tamoxifen has been shown to block a mild, non-lethal bone marrow hypocellularity provoked by oestrogens [42].

## Overview

Within the 'dark biology' of size the organotrophic system homeostatically regulates the masses of the tissues and organs of mammals in a manner involving the hypothalamic–pituitary–gonadal axis, the adrenals, thyroid and other endocrine tissues, growth factors, surface proximity molecules, the nervous system and so on, enigmatically integrated [43]. The system features both positive and negative control mechanisms. Among the positive influences are oestrogens, among the negative, a newly postulated endocrine entity of gonadal origin, micrin.

In female rats and ferrets endogenous oestrogens act mainly to upregulate soft tissue masses, both reproductive and non-reproductive. The evidence for this is that ovariectomy is moderately antiorganotrophic, while exogenous oestrogens are organotrophic. To cite the rat liver, oestrogens are regarded as co-mitogenic growth factors [44]. Oestrogens, endogenous and exogenous, influence tissue masses directly and also via effects at the hypothalamic–pituitary unit.

The micrin hypothesis states that in the rat there is a potent gonadal downregulator of tissue masses, both reproductive and non-reproductive. Ovariectomy exaggerates the organotrophic effects of exogenous oestrogens while blunting the antioestrogenic effects of clomiphene and tamoxifen, indicating a negative ovarian influence, micrin. In the intact male rat, a steroid-depleted putatively micrin-containing aqueous beef testicular extract shrinks the pituitary and prostate [29,45], while reversing, in a way that testosterone does not, the pituitary hypertrophy that follows castration [25]. These are among the observations and deductions supporting the existence of micrin (Table 1).

The micrin hypothesis meets the standard criteria of logical consistency, agreement with observation, economy of explanation and testability. Note that this hypothesis embodies a gonadocentric view of life. A key time for micrin influence, like that of oestrogen, could be at the onset of sexual maturity (puberty) and the growth spurt that goes with it, when a reduction in braking seems indicated (section "Reproduction").

A contention arising from the micrin hypothesis is that the full antiorganotrophic effects of clomiphene and tamoxifen involve not only oestrogen blockade, local and central, but also an increase in gonadal micrin. A second contention is that in the ferret, a seasonally monoestrous induced ovulator, clomiphene fails to block the organ-building effects of oestrogen, as it does in the rat, a year-round polyoestrous spontaneous ovulator, for two reasons: because a pituitary contribution to the oestrogen-induced organ weight changes is oestrogen-independent and because of the apparent unimportance in these effects of micrin. Oestrogens prob-

ably cause lethal bone marrow depression in ferrets because of a runaway pituitary–adrenal derangement unimpeded by an adaptive rat-like gonadal micrin 'brake'.

An objection to the micrin hypothesis is that it is based on data from drastic experiments in animals. Comparative endocrinology has traditionally been useful in tackling endocrine challenges [7, p. 14]. Specifically, organ ablation and hormone supplement studies in animals have proved valuable in untangling endocrine interactions involving the complex reciprocal relationship between the pituitary and gonads [46]. Even though the 'fit' between rat and human endocrinology is better than that between the ferret and human, the failure here fully to transpierce micrin endocrinology in the ferret as compared with the rat is an admitted weakness (Fig. 1). More knowledge will bring forth illumination.

Although the multi-milligram doses of oestrogens reported here appear excessive, they are not as unrepresentative as they might seem. Human ovarian follicular fluid can contain up to 1000 fold higher levels of oestrogen than blood serum [47]. During human pregnancy the production of oestrogens can reach 100 mg per day [48], which is 1–2 mg/kg. Furthermore, oestrogens still clearly retain their recognizable endocrinological character even when administered at the highest doses – and their actions can still be influenced by antioestrogens, in the rat at least. The organ weight changes under consideration do not represent anomalous high-dose threshold effects: oestrogens are broadly organotrophic and antioestrogens broadly antiorganotrophic at high and low doses. It can be asserted that what is being dealt with here are not 'toxic' effects, but an informative exaggeration of normal endocrinology.

Why has micrin not been described before? We will see later that it has, under a different guise (section "Is micrin a novel molecule?"), and that the need for the lifting of a 'brake' to usher in puberty has been conceptualised by others (section "Reproduction"). Suffice it to say here that considering the female, a fundamental difficulty is that organotrophic oestrogen and antiorganotrophic micrin emanate from the same organ, the ovary, vitiating the classical endocrine technique of organ extirpation, and both have effects centrally, the one (oestrogen) certainly, the other (micrin) hypothetically. Reproductive endocrinologists have often worked on ovariectomized animals to eliminate the confounding effects of endogenous oestrogens, eliminating ovarian micrin in the process. They have also concentrated their attention on classical target tissues such as the uterus and pituitary, missing the wider organotrophic and antiorganotrophic effects of oestrogens and antioestrogens, not least because of the low doses of active compounds used. In males the focus has been on pituitary, testes and prostate. Alternatively, researchers have used immature animals, again to eliminate the confusing effects of endogenous oestrogens. But juveniles respond differently to adults. For example, hypophysectomy in the mature female rat reduces the uterine growth reaction to oestrogens [4,19], but no such effect is seen in immature rats [49]. Testing in immature animals is thus insufficiently informative.

Hormonal toxicologists have adopted a more holistic approach than system-specific endocrinologists and have used the high doses needed to elicit easily observable changes. But they have undervalued information from oestrogen-related organ weight analyses because organ weight data, valuable in endocrinology, have contributed little to advances in toxicological understanding; because these data can be difficult to interpret due to body weight changes (though a careful protocol eliminates this problem, see section "Rat"); and because these data are simple to derive (using a balance) and thus lacking in molecular credibility. The provision of organ weight data in absolute terms (grams) in the early literature without relative data (e.g. % of body weight) is exasperating, given body weight variations. Under-analysis of hard-won organometric data is especially baffling, yet the (statistics free) data are often there in tables, although some of the old papers require textual sleuthing as to what was actually done.

The history of endocrinology is replete with examples of simple ideas that have proved enduring, even though daunting drifts of detail have subsequently piled up on them: sex/gonads, flight or fight/adrenaline, sodium/aldosterone, sleep/melatonin, etc. In this spirit is offered 'body's brake/micrin'.

## Is micrin a novel molecule?

Several criteria need to be fulfilled before an existing gonadal factor can be identified as the hypothesized micrin: (I) it must be antiorganotrophic (i.e. tissue shrinking) at the level of whole organs *in vivo* – if it does not do this it is something else; (II) it must act remotely, in the mode of a classical hormone, in this case in the body-wide organotrophic system, rather than just locally (though local production and activity is not precluded); (III) it must have as a main tissue of origin a ductless gland such as the gonad, again like a classical hormone, so that its effects can be revealed by organectomy (in this case, gonadectomy) – although this does not preclude other sites of production (section "Reproduction"); and (IV) it must be stimulated in terms of secretion or activity by the antioestrogens clomiphene and tamoxifen.

By dint of history and nomenclature the prime suspect for micrin has to be inhibin [7,8]. This is a dimeric gonadal polypeptide, existing in A and B forms, which downregulates the secretion from the anterior pituitary of follicle-stimulating hormone (FSH), without affecting luteinizing hormone (LH), while having paracrine effects on the gonads themselves [50]. Having the opposite action is activin, a related peptide. Follistatin is a further peptide which like inhibin suppresses FSH. Inhibin and activin are part of the transforming growth factor-beta (TGF-ß) superfamily of structurally related polypeptide growth and differentiation factors.

The term 'inhibin' was introduced by McCullagh [25]. Following work by others [23,51,52], McCullagh reported that castration in the male rat led to pituitary hypertrophy, also adrenal hypertrophy, as well as atrophy of secondary sex organs (prostate and seminal vesicles). McCullagh's recital of his inhibin's activities speaks of 'atrophy of the secondary sex glands [seminal vesicles and prostate] of normal male rats, a decrease in the amount of material ejaculated by guinea pigs and a cessation of the estrus cycle in female rats' [53]. In rabbits given aqueous testicular extracts there is reduced protein catabolism after castration, as indicated by a diminution of an expected rise in urinary creatinine [27].

The injection into castrated rats of extracts of bulls' testes prepared with fat solvents (e.g. benzene) reversed a loss of secondary sex characteristics and the atrophy of the prostate and seminal vesicles, but had no impact on the pituitary hypertrophy. The reversal effects were deemed to be due to the presence of the male steroid hormone 'androtin', later named testosterone. (Identified in 1935 as the mammalian sex hormone, testosterone in purified form was shown not to reverse the pituitary enlargement [26].) McCullagh found that the pituitary enlargement could however be eliminated by the administration of a steroid-depleted *aqueous* testicular extract, which he held to be due to a hormone which exerts an inhibitory influence on the pituitary, 'inhibin'. Intact male rats given an aqueous extract of bulls' testes show shrunken prostate and seminal vesicles. McCullagh was to say in a paper published in 1940 [54] that the 'most striking demonstration of this effect is due to Vidgoff' [45]. Indeed. As much inhibin as is in an aqueous extract of 9 kg of bulls' testes, delivered by subcutaneous injection to 20 normal rats for 28–35 days, knocked down the weight of the prostatic lobes of Vidgoff's rats by between a third and a half [45]. With this assay, subfractionation should have beckoned in the late 1930s, but no such procedure was reported. In fact

it was not until 1985 that a polypeptide was finally isolated, via molecular biology, and characterised as a gonadal downregulator of pituitary FSH. This was hailed as the 'inhibin' of yesteryear.

The first micrin identification criteria was 'antiorganotrophic *in vivo*'. As far as can be determined from the literature [7,8,55] no-one has injected modern-day inhibin into a castrated rat to reduce the pituitary enlargement that procedure causes. If modern-day inhibin does not display this activity, then it is not McCullagh's inhibin. Is there any reason to suppose that modern-day inhibin could shrink a castration-swollen rat pituitary? No. Castration cells probably appear in the pituitary as a result of an increase in the hypothalamic secretion of GnRH, judging from the experimental finding that immunoneutralization of GnRH by injection of antibodies causes the disappearance of these cells [24]. Inhibin does not affect GnRH secretion, acting instead at the pituitary. This means that removal of inhibin by castration should be without much consequence for hypothalamic function. de Jong commented on this discrepancy in a review [24, p. 557]. Remarkably, then, the castration experiment that launched the quest for a non-steroidal 'inhibin' does not seem to relate to the eventually discovered hormone at all.

The identification of modern-day inhibin with McCullagh's inhibin is particularly ironic given that McCullagh's special interest was prostatic hypertrophy. This is associated with testosterone, the testicular production of which is under the control of pituitary LH, unlike the ovarian production of oestradiol which also requires FSH [7, p. 1008]. Yet modern-day inhibin does not affect LH production, being a selective negative feedback regulator of FSH, which itself influences spermatogenesis. The adjustment of what constitutes 'inhibin', from something the absence of which causes pituitary hypertrophy after castration to FSH downregulator, seems mainly attributable to Klinefelter in the early 1940s [56]. In the castrated rat there is an increase in pituitary LH as the frustrated pituitary calls for more testosterone from the absent testes. If this rat shares a circulation with a normal intact male, in celio-anastomotic parabiosis, the prostate of the normal male will enlarge considerably [53]. This is because the LH from the castrate animal stimulates testosterone production from the normal testes, which boosts the prostate. (This is also true of the castrate's partner if that animal has been hypophysectomized [57].) If McCullagh's inhibin is injected into the castrate, the normal animal's prostate will atrophy [53], because the castrate's pituitary LH secretion is suppressed, ultimately reducing testosterone in the normal animal. In principle it is impossible that Klinefelter's FSH-downregulating modern-day inhibin could accomplish such a feat.

McCullagh's inhibin causes prostatic atrophy in the rat when ingested in the form of a whole desiccated beef testicular preparation [58,59]. A polypeptide such as modern-day inhibin is unlikely to be orally active. McCullagh's inhibin, in the form of a desiccated beef testicular extract, was used in the 1930s to treat human benign prostatic hyperplasia (BPH), with some success in terms of symptomatic relief (section Prostate). It is hard to see why injections of modern-day inhibin would be useful in BPH, as important as this FSH downregulator might be in the second rank of reproductive hormones.

We can conclude that modern-day inhibin is not McCullagh's inhibin. Is modern-day inhibin nonetheless micrin? Mice from which the gene for modern-day inhibin has been knocked out are sterile and sport gonadal tumours [55]. They die of a wasting condition due to unopposed and increased activin signalling, as inhibin is an activin receptor antagonist. This portrays inhibin as an anti-downregulator or *de facto* upregulator, the absence of which leads to reduced viscera. Micrin, on the other hand, is a postulated downregulator. The predicted phenotype of a micrin knockout mouse would involve visceromegaly, possibly accompanied by adrenomicria. This indicates that modern-day inhibin is not micrin. If modern-day inhibin were micrin, then generations of inhibin researchers have failed to notice their molecule's therapeutic potential (section "Therapeutic opportunities").

The strategy to find McCullagh's inhibin would be a fractionation campaign, using an aqueous extract of gonadal starting material, guided by a bioassay based on the castrated rat. The simple endpoint would be shrinkage of the castration-swollen pituitary. This is also the strategy that can be recommended in the quest for micrin. This discloses that McCullagh's inhibin and micrin are one and the same entity.

If a gonadal entity reduces the enlarged pituitary of castration, it is likely to be micrin; if not, not. The desideratum is to stay firmly *in vivo* and focus on organ size *per se*, via an organometric assay, without substituting functionality (e.g. modulation of GnRH or FSH) or heading off into a proxy study *in vitro* (e.g. of cell proliferation). In organometrics size is not a proxy for anything else: it is the message.

Relaxin is another ovarian hormone. This pregnancy-related peptide promotes symphyseal relaxation, softens the cervix and suppresses spontaneous myometrial contractility. Its production is indirectly stimulated by oestrogen [60], while not being much affected by clomiphene [61]. This, and the fact that relaxin is uterotrophic not anti-uterotrophic, eliminates this candidate. The uterotrophic effect of relaxin can be blocked by progesterone [62]. This latter hormone can also reduce the uterotrophic effects of oestrogens, through a suppressive effect on the numbers of uterine oestrogen receptors. The secretion of progesterone is unaffected by clomiphene [63]. This is against progesterone being micrin, even without the early work in the male showing that McCullagh's inhibin (i.e. micrin) is not a steroid. Furthermore, progesterone treatment in rodents results in an anabolic effect on non-reproductive tissues and organs, not the reverse [64]. Testosterone, another ovarian steroid, is stimulated by clomiphene [65], but like progesterone is anabolic, notably in regard to muscles.

Myriad messages in fact emanate from gonadal cells [7, p. 1351;8,61]. Among proteins and peptides endocrine effects have only been demonstrated clearly for modern-day inhibin [7, p. 1358]. Most gonadal entities seem to be involved in paracrine and autocrine regulation and most are upregulators. On the basis of the identification criteria, there is no prima facie case for supposing any to be micrin.

If the co-identification of McCullagh's inhibin and micrin is valid, then something can be learned of the chemical character of micrin from the work in the 1930s. Vidgoff's group [45] subjected bull's testes from an abattoir to physical maceration, then to a protein precipitation step using sodium sulphate, the precipitated protein being captured by a filter. The resulting 'thick juice' was then chilled to provoke sulphate crystallisation, the crystals being removed by another filter. Benzene was added to the sulphate-depleted filtrate in twofold excess, to remove steroids, with the benzene layer being discarded. The remaining aqueous fraction was then concentrated by evaporation, for administration to normal testes-intact rats via subcutaneous injection, shrinking their prostates by between a third and a half. Note that an alternative to the removal of sulphate by crystallisation and filtration was tried in the form of alcohol precipitation, but this technique led to loss of activity. That McCullagh's inhibin is orally active, supplies a further piece of information.

It is probably safe to say that the active principle is nonsteroidal. It may be nonproteinaceous as well, but this is less certain, as salt precipitation and the other procedures will not remove or destroy all proteins. It can be asserted though that no macromolecule is likely to have survived the Vidgoff process in its native state. A peptide fragment of a larger protein might get through, bearing some of the activity of the native molecule and displaying oral activity. Exposure to sodium sulphate would likely put the pep-

tide's C-terminus into a charged state ($COO^-$ $Na^+$), enhancing its aqueous solubility. Excess alcohol could then engender C-terminal ester formation ($COOC_2H_5$), rendering the peptide more hydrophobic and thus potentially removable by alcohol solubilisation. Or the C-terminal esterification could compromise activity, even if the peptide were not removed. This line of argument speaks of an organic acid, whether peptidic or not. Oral availability might suggest a small molecule.

Assisting in a micrin isolation campaign is apparent knowledge of site of production. The 'inhibin'-defining pituitary hypertrophy seen in rats after castration is also seen after X-ray sterilisation or autotransplantation of the testes to the excessively warm abdomen ('cryptorchidism'). These procedures destroy the germinal epithelium of the testes but not the interstitial cells which make testosterone [27,66].

The biology of size and shape describes influences extrinsic (e.g. nutrients, crowding) and intrinsic. Among the latter are patterning gradients of morphogens, insulin/IGF signalling, fruit fly gene and protein mechanisms with actual or probable mammalian homologues, organ-specific size sensing, planar polarity (i.e. 'up') awareness, proximity factors, cell competition and so on. But withal there is a seeming insufficiency of inhibition, of stop signals, especially at long range. Enter micrin, as first sighted in 1990 by the author as an inhibitory ovarian influence that 'requires characterisation' [1].

## Therapeutic opportunities

The rat tends to be a good model for the human in terms of endocrinology, notably female reproductive endocrinology [12]. Clomiphene and tamoxifen are effective as antioestrogens in the human as they are in the rat but not the ferret (Fig. 1). This observation alone suggests that the organ-mass determining organotrophic system in humans is indeed more like that of the rat, another spontaneously ovulating polyoestrous animal, than that of the monoestrous ferret, a seasonally breeding induced ovulator. Specimen applications only are picked out here for micrin agonists and antagonists, from a potentially long list which would include polycystic ovary syndrome, endometriosis, pituitary adenoma, cardiac hypertrophy, osteoporosis, wound healing, drug-eluting stents, organ regeneration, etc.

### *Reproduction*

A pituitary extirpated from a mature rat and transplanted into an immature one will induce precocious sexual maturity in the latter. Surprisingly, the same effect is seen if the transplanted pituitary is taken from a sexually immature rodent and inserted into another sexually immature rodent, even if the transplant does not 'take' or comprises pre-killed pituitary cells [67]. There is presumably enough gonadotropin in the immature pituitary to support puberty, but it is held in check in the immature animal. There is evidently a brake.

Castration in the rat causes pituitary hypertrophy due to a deficiency of McCullagh's inhibin (micrin). This effect is brought about by an increase in hypothalamic GnRH (section "Is micrin a novel molecule?"). Pituitary enlargement after castration asserts itself in 2–3 weeks at 7 days of age, but 2–3 months in adulthood [27]. This implies a slackening of micrin braking after sexual maturation. As an increase in GnRH is the hallmark of puberty, a reduction in micrin braking when an appropriate pattern of organ sizes is attained can be conjectured to be involved in the onset of sexual maturation. This is puberty induction via hypothalamic disinhibition.

Consistent with reduced micrin braking prompting sexual maturation and the associated achievement of full adult stature is the observation that the administration of semi-purified McCullagh's inhibin (micrin) decreases growth rate in rats [58,59].

Gonadarche is the pubertal activation of the gonads in the direction of gametogenesis and steroid synthesis [7,8]. It is preceded by adrenarche, which comprises maturation of the adrenal cortex, with increased secretion of adrenal androgens, leading to the appearance of sexual hair (pubarche) [8 p. 377]. What initiates adrenarche is unknown, but the involvement of micrin can be suspected. The process will probably not be one of adrenal disinhibition, however, because organometric studies in rats indicate that micrin is adrenotrophic not inhibitory (section "Rat"). A prepubertal micrin surge before a decline might be involved.

So far the model is of the gonads activating themselves at puberty via a reduction in gonadal micrin. We can transcend this anomalous model by considering the process further. Gonadarche is triggered by 'a resurgence in the activity of the hypothalamic GnRH pulse generator that has been held in check by a neurobiological brake (viewed to be conceptual in nature) from the time of late infancy. Current models posit that the GnRH pulse generator is comprised of KNDy neurons in the arcuate nucleus of the hypothalamus, and that the output of the pulse generator is relayed to the GnRH neuronal network by the intermittent release of [stimulatory] kisspeptin. The components of the neurobiological brake that dictates the postnatal pattern of GnRH pulse generator activity remain a mystery' [8 p. 420].

The mystery of what starts gonadarche can be addressed as follows. Apart from a non-shrinking adrenal, the organ reductions of high-dose clomiphene treatment [4] resemble in extent the splanchnomicria (small viscera) seen after hypophysectomy rather than the mild organ regressions of ovariectomy. This invites the conjecture that there is a hypothalamic 'organostat' controlling tissue and organ masses and involved in the 'decision' to commence puberty. This would be analogous to the appestat which controls appetite and the other hypothalamic centres controlling body temperature, respiration and many other functions [7,8]. Lesioning discrete hypothalamic nuclei should find one at least that alters organ weights on a body-wide basis. An economical explanation for the antiorganotrophic effects of clomiphene is that it acts in the same way at the hypothalamus as at the gonad: through (well-attested) oestrogen receptor antagonism and (hypothesized) micrin induction. The suggestion here is that while the gonads provide endocrine (circulating) micrin, the hypothalamus and perhaps other higher centres indulge in de novo micrin production for local (paracrine) consumption, as part of a somatic cueing system for puberty.

The foregoing predicts that the administration of exogenous micrin will reduce human reproductive potential. Conversely, micrin antagonists and micrin-neutralizing antibodies might be of clinical and veterinary interest in the context of infertility.

### *Breast*

Breast cancers are often dependent for their growth on oestrogens, which is held to account for the clinical finding that about a third of patients respond to treatment with an antiestrogen such as tamoxifen [7,8]. Oestrogen receptors can be detected in 70% of primary breast cancers [7, p. 1611]. Absence of oestrogen receptor in the tumour predicts that less than 5–10% of women will respond to this therapy. That any patients in this category respond implies that blockade of tumour-located oestrogen receptors cannot be the sole basis of tamoxifen's efficacy. Multiple cellular mechanisms have been proposed by which tamoxifen is an off-target chemotherapeutic, i.e. is effective in ways not involving the oestrogen receptor [68,69]. Clomiphene has also been shown to have efficacy

in breast cancer [15]. It was hypothesised above that this compound is antiorganotrophic in the rat in part due to its ability to compete for the oestrogen receptor and in part due to an increase in the ovarian secretion of the hypothetical organ-mass inhibitor, micrin. Clomiphene and tamoxifen are probably successful in the treatment of breast cancer for the same two main reasons.

Tamoxifen is more effective in breast cancer postmenopausally than premenopausally [70]. There is more oestrogen to oppose premenopausally, of course, so this result might be regarded as surprising. Even if oestrogen receptor protein is undetectable in the primary breast tumour of a postmenopausal woman, tamoxifen can still be effective in reducing risks after surgery [71]. A shift can be suspected at the menopause in the oestrogen:micrin ratio in favour of micrin. There is evidence for this (section "Ageing"). Tamoxifen, a putative micrin inducer, may be making a favourable ratio more favourable. This makes the case for the use of exogenous micrin in breast cancer.

*Prostate*

The incidence of prostate diseases increases with age, such that by 80 years 70% of men have detectable prostate tumours at autopsy [7, p. 1622]. Of diagnosed prostate cancers, 80–90% are androgen receptor positive at diagnosis and treatment usually includes androgen deprivation in the form of orchiectomy (castration), actual or chemical, the latter approach involving the use of GnRH analogues and antiandrogens [7,8].

The cause of benign prostatic hyperplasias is an enduring enigma, but it is related to the action of testosterone and especially to its more androgenic metabolite dihydrotestosterone. Drug treatment therefore tends to involve inhibiting the enzyme that converts testosterone to dihydrotestosterone.

McCullagh in his 1932 inhibin paper [25] speculated that 'If the testicular cells producing "inhibin" were to fail, previous to the failure of those structures which produce "androtin," [i.e. testosterone] the hypertrophic phenomenon could easily be explained. The absence of "inhibin" would result in hyperfunction of the pituitary gland which. . .is a known cause of prostatic hypertrophy in rats.' McCullagh's inhibin is not modern-day inhibin, the gonadal downregulator of pituitary FSH, but what is being referred to in the present paper as micrin (section "Is micrin a novel molecule?"). McCullagh's schema is that the pituitary, freed from testicular restraint, goads the testes into producing excess testosterone, giving rise to prostatic enlargement [66]. Yet we now know that absolute levels of androgen are not of the first importance: androgen levels decline with age, at a time when prostatic problems are most prevalent, and exogenous androgen is not associated with increased prostate disease [7, p. 1479].

Bovine testicular mush causes prostatic hypertrophy by injection, but prostatic atrophy by ingestion [58]. This effect depends on the poor oral availability of prostate-boosting testosterone, which taken orally in unmodified form is rapidly inactivated by first-pass hepatic metabolism [7, p. 1124], versus the oral availability of prostate-shrinking McCullagh's inhibin (micrin), achieving a natural fractionation. In the early 1930s a nearly two-thirds reduction in prostate size was seen in rats fed 0.2 g of desiccated beef testis (i.e. less than a thousandth of their body weight) 5 days in seven for about 100 days, compared with controls, with health maintained [58].

An aqueous extract of bovine testes, representing McCullagh's inhibin, was used to treat BPH patients in 1930s North America with 'good results' and there also seems to have been successful work in France in the period on similar material [72]. McCullagh's experimental testbed was the rat, which is a plausible model for the human male as the prostate gland in both species enlarges with age. The relative weight of the aged rat prostate is two-thirds bigger than that of the midlife prostate [59]. McCullagh's clinical material was a steroid-depleted aqueous extract of bull's testes, subject to desiccation and administered orally for 30 days or so [73,74]. Symptoms were relieved in a significant proportion of patients in regard to urination difficulties arising from obstruction at the neck of the bladder. An increase in bladder muscle tone was suspected, as bladder emptying was achieved satisfactorily in the apparent absence of a change in size of the prostate [74]. The comment was made [73]: 'It is obvious that as we have not fractionated the product and obtained the active principle, general application is not practical.'

To 'micrinise' McCullagh's schema: if the testicular secretion of micrin, the organotrophic 'opposition' to androgen, falls faster than the decline in testosterone with age, then there would indeed be the condition for prostatic overgrowth. McCullagh regarded pituitary suppression as his inhibin's main activity. As the same active principle, micrin has this property but additionally micrin, conceived of as widely antiorganotrophic, can be postulated to be directly antiprostatic and directly active on the bladder too. Within this interpretive framework, BPH becomes a micrin deficiency disorder, resulting from a fall in the ratio of micrin:androgen. The treatment for this condition and for prostatic carcinoma as well? Hormone replacement therapy for men, using micrin. Clomiphene could be considered as a prostate treatment, as a putative micrin stimulator, but might be problematic given its testosterone-raising capability [65]. A micrin-related drug seems preferable, possibly in combination with an androgen-reducing drug.

*Bone marrow*

The fatal aplastic anaemia induced by oestrogens in ferrets and dogs occurs in the apparent absence of an adrenal-related micrin braking effect seen in rats (section "Ferret"). If micrin is protective of the bone marrow, then this would be a paradoxical property for a tissue-shrinking hormone and an extremely useful one for an entity which could form the basis of a new class of anti-tumour agents. Not only can use on their own be visualised for micrin-based drugs but also co-administration with cytotoxic agents, since bone marrow is the dose-limiting tissue for most of the latter. Exogenous micrin might be helpful generally in conditions of haematopoietic insufficiency.

*Ageing*

Micrin endocrinology is relevant to ageing, a complex and poorly understood process [7, p. 1483, 8] that may involve a hypothalamic age clock [75]. In male rats gonadectomy provokes pituitary enlargement far more quickly in young immature animals than in adults [27]. This yields a picture of an age-related decline in pituitary inhibition by the testes, hypothetically attributable to a declining ratio of micrin:sex steroids. In line with this falling ratio is the ballooning of the prostate seen in older rats and men (section "Prostate"). As there are age-related reductions in bioavailable testosterone and oestrogen in human males [76], micrin must decrease even more in absolute terms.

In the young adult female rat gonadectomy decreases the relative size of the liver [77], for example, and to a lesser extent that of the kidneys, within a generally antiorganotrophic picture, which includes pituitary shrinkage (section "Rat"). The loss of a net positive ovarian influence is indicated, implying the predominance of oestrogen (and testosterone) over micrin in young adult female rats. To investigate the response to ovariectomy in older female mammals the author undertook a study with colleagues in aged sheep at Harwell Laboratory, UK. Six multiparous non-pregnant ewes of 7–13 years were assigned semi-randomly to two groups, such that the mean ages and body weights of the two groups were

similar. One group was subject to surgical ovariectomy, the other to sham ovariectomy. At termination 18 months later the mean relative kidney weights (% body weight) of the ovariectomized sheep were a non-significant 10% higher than those of sham operated controls, while the mean relative liver weights were 29% above those of controls ($P < 0.01$). The removal of antiorganotrophic ovaries was indicated.

The mammalian ovary thus may progress from being a net organotrophic influence in young adults to a net antiorganotrophic influence when old. This implies that a micrin:sex steroids ratio favouring sex steroid in females is superseded by one favouring micrin. This is in contrast to the declining ratio of micrin:sex steroids inferred for older males. Older women appear to be getting more endogenous micrin protection against tissue overgrowth than older men and, conjecturing, perhaps this 'micrin plus' status helps to keep them alive longer than their men folk. Some men are regarded as worth treating with testosterone, as having too little [7,8], but almost all men are 'micrin minus', with an expanding prostate as the prime indicator. So as well as shrinking the prostate, supplementary micrin might boost male longevity, narrowing the sex gap in lifespan. In absolute terms the body's brake may weaken in both sexes over time, contributing to a rise in cancers with age.

## Conclusion

The present paper has privileged size over function and morphology to illuminate the mammalian internal-size-regulating 'organotrophic system'. The key to this system is asserted to be the hypothalamic–pituitary–gonadal axis, in part in the form of organotrophic gonadal sex steroids, balanced by a postulated antiorganotrophic hormone also of gonadal origin, micrin, the body's brake against tissue overgrowth. At the top of the axis there is conjectured to be brain micrin as well, whose paracrine (local) inhibitory effect is lifted at an appropriate aggregate organ mass, propelling the organism to sexual maturity.

## Conflict of interest

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