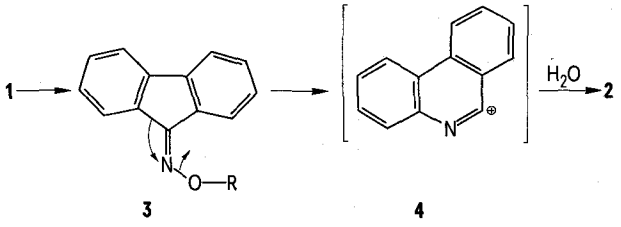
formation of the amide. Furthermore, such processes are generally concerted[10], so that if the metabolic reaction proceeds via a similar mechanism, electrophilic intermediates such as those generated in reactions following arylhydroxylamine esterification[9] and considered responsible for eliciting toxic or carcinogenic responses would not be expected to be formed.




3 4

Where R may = $PO_3^{2-}$
glucuronide
$-SO_3$
O
||
R′–C–

Pan and Fletcher[11] have reported that substituted fluorenone oximes exhibit anti-neoplastic activity, as indicated by their effect on Walker carcinosarcoma 256 and L-1210 leukemia. Parallel activity was found with the corresponding phenanthridinones, synthesized from the oxime by reaction with polyphosphoric acid. It now appears that this parallel activity between these structural isomers may not be fortuitous, but rather results from metabolic rearrangement of the oxime. This possibility is being investigated.

10 T. S. Stevens and W. E. Watts, in: Selected Molecular Rearrangements, p. 52. Van Nostrand Reinhold Co., London 1973.
11 H. Pan and T. L. Fletcher, J. med. Chem. *12*, 822 (1969).

Table 1. TLC Identification of phenanthridinone as a metabolite of fluorenone oxime

| Stationary support | Solvent system | $R_{f\,oxime}$[a] | $R_{f\,amide}$[b] |
|---|---|---|---|
| Silica gel[c] | Ethyl acetate:methanol: ammonia [85:10:5] | 0.88 | 0.80 |
| Alumina[d] | Chloroform:methanol [9:1] | 0.05 | 0.29 |
| Polyamide[d] | Chloroform:methanol [9:1] | 0.60 | 0.70 |

a) Fluorenone oxime detected fluorometrically. b) Phenanthridinone detected fluorometrically. c) E. Merck, Darmstadt, W. Germany. d) Eastman Chemical Co., Rochester, N.Y.

Table 2. Spectral properties of metabolites of fluorenone oxime

| Property | Characteristics |
|---|---|
| UV[a,b] | $\lambda_{max}$ 337 nm and 323 nm |
| Fluorescence[a,b] | $\lambda_{ex}$ 323 nm; $\lambda_{em}$ 360 nm<br>$\lambda_{ex}$ 337 nm; $\lambda_{em}$ 360 nm |
| Mass spectrum[c] | Major bands:<br>m/e<br>149 (6.3)[d]<br>135 (26.7)<br>120 (1.3)<br>93 (100) |

a) All spectral characteristics of the metabolite corresponded to the properties of authentic phenanthridinone. b) Spectra recorded in chloroform:methanol (9:1) solution. c) All spectral bands with intensity equal to or greater than 1% of the base peak corresponded to the spectrum of authentic phenanthridinone which had been similarly chromatographed. d) % of relative abundance of base peak in parentheses.

## The effect of clomiphene citrate and estradiol on body weight, vaginal cornification, and uterine weight after chronic treatment of ovariectomized rats[1]

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*Summary.* Clomiphene reduced the body weight gain of ovariectomized rats to a much greater degree than extradiol did. Estradiol had a more pronounced effect on vaginal cornification and uterine weight than clomiphene did.

Clomiphene citrate (Clomid®) is one of many antiestrogens which possess weak estrogenic potency[3,16,17] and have various effects on the female reproductive system[3,4,14,15]. One of the problems in evaluating the mechanism of action of these compounds is comparing them to the effect and potency of the natural estrogens. Depending on the parameter and dose, these antiestrogens may be more, less, or as effective as estradiol[4,5]. In addition, some of the antiestrogens appear to have effects which are quite different from those of estradiol[5,6]. One effect of estradiol is to reduce the b.wt gain observed in ovariectomized rats[7]. One series of antiestrogens was reported to be far more effective than estradiol in reducing rat b.wt gain despite its weak uterotrophic activity[8].

We have previously reported that clomiphene was far more effective than estradiol in increasing luminal epithelial glycogenesis in rat uterus[6]. One dose of clomiphene was less effective than estradiol in increasing glycogen concentration but 3 doses of the drug were as effective[9]. Because of the marked difference in the effects of clomiphene and estradiol on one uterine system, it was of interest to investigate the effects of chronic treatment on b.wt gain and 2 other sensitive indices of estrogenic potency-vaginal cornification and uterine wt. The experiment was conducted in order to investigate further the comparative estrogenicity of clomiphene and estradiol, and to determine if clomiphene followed the pattern of other antiestrogens which have a greater effect on b.wt than does estradiol.

*Materials and methods.* Virgin female Holtzman rats were housed 3 per cage under controlled lighting (14 h light) and temperature (22 °C) conditions and allowed free access

to food and water. The rats weighed 180–200 g at the time of ovariectomy. The rats were bilaterally ovariectomized under ether anesthesia, rested for 4 weeks, and divided into 3 groups of 5 animals. Group 1 was the ovariectomy control and received the clomiphene vehicle (5% acacia). Group 2 received 1.0 µg estradiol dispropionate/rat/day s.c. in cottonseed oil. Group 3 received clomiphene citrate (2.5 mg/kg b.wt/day) by gavage. The rats were treated once/day for 15 consecutive days and killed by cervical dislocation 24 h after the final treatment. Light ether anesthesia was used to facilitate accurate b.wt recording and daily treatment. Daily b.wt and vaginal smears were recorded. At autopsy the uterine cornua were removed, trimmed of excess fat and mesentery, and weighed. One piece was placed in an oven (100 °C, 24 h) for dry weight determination. The data were analyzed by Duncan's New Multiple Range Test.

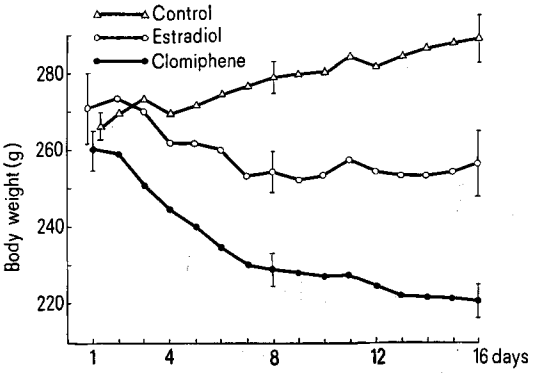
*Results.* The daily b.wt (figure) were not recorded during the postovariectomy period. The rats gained about 60 g between ovariectomy and the first treatment day. The ovariectomized controls continued to gain weight throughout the treatment period. Estradiol caused a small but significant weight loss through the first 7 days which was followed by a plateau. Clomiphene caused a marked weight loss which was quite striking over the first 7 days and also appeared to plateau. The clomiphenetreated animals did not appear chronically ill.

The effect of the drug and hormone on the uterine wt is shown in the table. Estradiol caused a much larger increase in both wet and dry weight than did clomiphene. The effect of the 2 compounds on relative uterine wt paralleled that on the absolute uterine wt increase. The uteri of the estradiol-treated rats appeared more hyperemic than those of the clomiphene-treated animals.

The effect of clomiphene and estradiol on relative (mg/kg) b.wt and absolute (mg) uterine wt. The data is expressed as mean ± SE

| Treatment | Rats | Absolute wet | dry | Relative |
|---|---|---|---|---|
| Control | 5 | 99.8 ± 11.9 | 20.1 ± 2.1 | 0.35 ± 0.04 |
| Clomiphene | 5 | 138.2 ± 4.8* | 27.9 ± 1.6* | 0.63 ± 0.02* |
| Estradiol | 5 | 297.3 ± 12.4** | 55.9 ± 3.8** | 1.16 ± 0.10** |

*$p < 0.05$ vs control; ** $p < 0.01$ vs control and clomiphene.




The effect of daily doses of clomiphene and estradiol on total b.wt. The bars on days 1, 7, and 16 represent SE of the mean. There was a significant difference in the 3 groups on day 7 and thereafter ($p<0.01$).

All the rats in both the clomiphene and estradiol groups had cornified vaginal smears by the morning of the fourth treatment day. However, the groups were quite different on the subsequent 12 days of the experiment. Estradiol-treated rats exhibited a persistent estrus smear with cornified cells predominant for the last 12 days. After the fourth day each of the clomiphene-treated rats had a different daily smear pattern which was quite variable. These 5 animals had typical estrus type smears for only 1, 5, 7, 8, and 10 days, respectively, versus the 12 days of the estradiol group. Various combinations of the 3 cell types (cornified, nucleated, and white blood cells) were observed on the other days.

*Discussion.* The most significant results of this experiment were those showing the marked weight reduction in the clomiphene-treated animals. Such an effect has been reported for other antiestrogens[8]. Estrogen is known to cause a reduction in b.wt[7]. Clomiphene was clearly more effective than estradiol in reducing b.wt. However, it was not clear from the present study whether clomiphene's mechanism of action was similar to estradiol's or not. Estradiol may act through its effect on the hypathalamus[10]. Clomiphene has also been shown to effect hypothalamic function[3,11,12], and it is reasonable to speculate that the effects may therefore be similar.

The effects of clomiphene and estradiol on vaginal smears were quite different from their effects on b.wt. The hormone was far more effective than clomiphene in inducing vaginal cornification and persistent estrus. Although clomiphene was able to induce vaginal cornification, the effect was not maintained throughout the treatment period. This is consistent with other reports of clomiphene's weak estrogenicity[13].

Other investigators have reported that clomiphene is more effective than estradiol in increasing uterine weight[11,12,14,15]. However, 3 daily doses of clomiphene and estradiol caused similar uterine wt increases[9]. The present data showed a distinct difference since clomiphene caused only a 37% increase while estradiol caused 197% increase over control values. Obviously there is a significant difference in the time and dose-related effects of the

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3 R. B. Greenblatt, in: Progress in Infertility, p. 455. Little, Brown and Co., Boston 1968.
4 J. A. Cidlowski and T. G. Muldoon, Biol. Reprod. *15*, 381 (1976).
5 P. K. Mehrota, J. N. Karkun and A. B. Kar, Contraception *7*, 115 (1973).
6 W. L. Poteat and W. J. Bo, Anat. Rec. *169*, 717 (1971).
7 M. F. Tarttelin and R. A. Gorski, Acta endocr. *72*, 551 (1973).
8 G. Herbai, Acta endocr. *68*, 249 (1971).
9 W. L. Poteat, unpublished data.
10 G. N. Wade and I. Zucker, J. comp. physiol. Psychol. *72*, 328 (1970).
11 A. J. Eisenfled and J. Axelrod, Biochem. Pharmac. *16*, 1781 (1967).
12 S. Roy, R. B. Greenblatt and V. B. Mahesh, Acta endocr. *47*, 657 (1964).
13 J. W. Ross, J. Shryne, R. A. Gorski and J. R. Marshall, Endocrinology *92*, *1079* (1973).
14 J. R. Wood, T. R. Wrenn and J. Bitman, Endocrinology *82*, 69 (1968).
15 S. Mohla and M. R. N. Prasad, Steriods *11*, 571 (1968).
16 M. S. Sankaran and M. R. N. Prasad, Gynec. Invest. *3*, 142 (1972).
17 L. Terenius and I. Ljungkvist, Gynec. Invest. *3*, 96 (1972).

2 compounds. This dose of clomiphene was clearly not effective enough to maintain the uterotrophic effect it had at 3 doses nor to extend vaginal cornification throughout the treatment period as estradiol did. It appears that clomiphene was estrogenic enough to mimic the early effects of estradiol but not to maintain those effects over a long period.

The results add further evidence to the reports[4,5,8] that generalizations concerning the relative estrogenic effectiveness of synthetic antiestrogens should be viewed with caution. These agents which possess at least weak estrogenicity may be as effective or even more so than estradiol in influencing the various indeces of estrogenic sensitivity. Clearly, clomiphene is one of those.

## Adrenergic supersensitivity of the pupil in idiopathic headache[1]

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*Summary.* In idiopathic headache (IH) sufferers, phenylephrine and fenfluramine induce a pupillary dilatation respectively greater and lesser than in controls. The difference may be due to a supersensitivity of the iris alpha adrenoceptors caused by a deficiency of noradrenaline in the iris adrenergic nerve terminal of the IH sufferer. These findings seem to support the hypothesis of a brain receptorial, monoamine supersensitivity in IH.

Idiophatic headache (IH) could be due to an impairment of the central modulation of pain[2]. A deficiency of brain monoamines, 5-hydroxytryptamine in particular, and consequently a supersensitivity of the peculiar postsynaptic receptors, has been suggested to be the main biochemical disorder in IH[3].

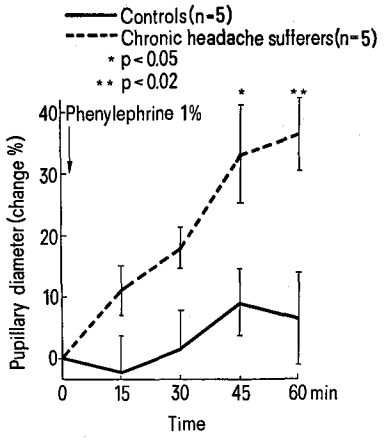


Fig. 1. Pupillary dilatation after phenylephrine 1% instilled in the right conjunctival sac. Each point represents the mean ± SE; *, **, significantly different from the control group.

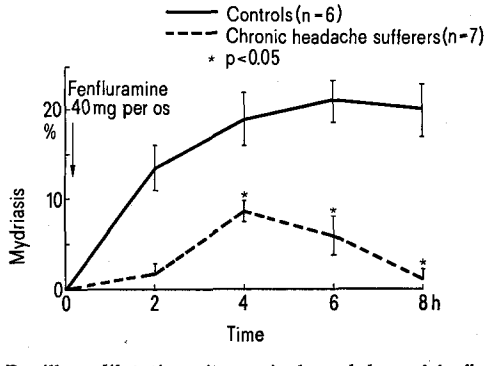


Fig. 2. Pupillary dilatation after a single oral dose of fenfluramine. Each point represents the mean ± SE; *, significantly different from the control group.

Since the pupil innervation is considered as an emanation of the brain, it is therefore possible that the pupil reactivity provides indirect information concerning the monoamine turnover and receptorial sensitivity of the central nervous system.

*Materials and methods.* 12 drug-free volunteers, sufferers from chronic headache, were studied. Phenylephrine 1% was instilled in 5 of the patients (3 women and 2 men with a mean (± SE) age of 40 (± 19) years, ranging from 35 to 46) in the right conjunctival sac. The remaining 7 patients (3 women and 4 men with a mean (± SE) age of 30 (± 40) years, ranging from 17 to 48) received 40 mg of fenfluramine hydrochloride in a single oral dose.

The control group was composed of 11 healthy drug-free volunteers. 5 controls (2 women and 3 men with a mean (± SE) age of 26 (± 22) years, ranging from 21 to 32) received phenylephrine. The fenfluramine control group was composed of 3 women and 4 men (with mean (± SE) age of 32 (± 18) years, ranging from 20 to 42).

The pupil diameter was always studied in the same light intensity by using the photographic technique of Sneddon and Turner[4,5] with some modifications[6]. The pupil was measured before the drugs; 15, 30, 45 and 60 min after phenylephrine and 2, 4, 6 and 8 h after fenfluramine. Differences between pre- and post-drug values and between headache and control groups were compared by using a trained Student's t-test.

*Results.* a) Phenylephrine instillation did not induce any statistically significant pupillary dilatation in controls. In IH sufferers, it provoked a statistically significant mydriasis which lasted for at least 60 min. The p-value of the difference between pre-drug and 15, 30, 45 and 60 min after phenylephrine was 0.05, 0.01, 0.02 and 0.005 respectively. The difference between the control and headache group was statistically significant 45 and 60 min after the eye instillation (figure 1).

1 This study was supported by a grant from the National Research Council, Rome, Italy.

2 F. Sicuteri, Pharmac. Res. Commun. *3*, 4 (1971).
3 F. Sicuteri, B. Anselmi and P. L. Del Bianco, Psychopharmacologia, Berl. *29*, 347 (1973).
4 J. M. Sneddon and P. Turner, J. Physiol., Lond. *1189*, 20 (1967).
5 P. Turner, Drugs and the special senses, Seminars in drug treatment. *1*, 335 (1971).
6 P. Galli, M. Fanciullacci, G. Monetti and E. Assenza, Boll. Soc. ital. Biol. sper. *52*, 1948 (1976).