vity within 20–60 sec. (The times are upper limits because of the need to scan the entire plate.) The replacement of the medium with fresh medium did not cause an immediate resumption of activity, but all cultures showed weak fibre contractile activity after 70–80 min at 37 °C. Full strong activity was observed after 4 h at 37 °C. No other changes were noted on further incubation.

Lower concentrations of diamide ($1–2 \times 10^{-4} M$) were without appreciable effect upon the contractile activity of the fibres in the cultures. Higher concentrations ($3 \times 10^{-3} M$) of diamide caused an instantaneous loss of contractile activity, observed through the microscope during the addition of the reagent.

The specificity of our thiol-oxidizing agents has been detailed elsewhere[1,2,5]. The rapid cessation of contractile activity in muscle fibres after intracellular oxidation of glutathione to the disulphide implies a close and possibly direct role for GSH in the contractile activity. Further support for this role is found in the length of the time for recovery of contractile activity, a time reasonable for the intracellular regeneration of GSH from GSSG[2]. The spontaneous contractile activity of muscle fibres in culture may bear a close relationship to the acetylcholine stimulated activity in denervated muscles[8,9]. The molecular basis for the participation of GSH in muscle contraction is under investigation.

Résumé. Le traitement de fibres en pulsation d'un muscle (cultivé en vase clos) avec l'oxydant diamide, spécifique pour la conversion du glutathione (GSH) au disulfide (GSSG), arrête vite tout mouvement. Après quelques heures d'incubation, le niveau normal de l'activité est retabli.

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Effects of Cyclohexylamine on Rat Fertility

The biological effects of cyclohexylamine, the major degradation product of cyclamate, on chromosomes, reproduction and teratogenesis have been the subject of several papers[1–4]. In an earlier communication, a deleterious influence of cyclohexylamine sulphate on male fertility was described[3]. Later investigations showed that females mated with cyclohexylamine sulphate-treated males had smaller litters at term than those bred with controls. The finding was suggestive of genetic damage and forms the subject of this report.

Male Wistar albino rats weighing 175–200 g were randomly assigned to test (15 animals) and control (10 animals) groups. The test males were fed 0.2% cyclohexylamine sulphate (CHS) in their drinking water. At the same time, a 65-day breeding program was initiated in which each male was isolated with 2 virgin females for 5 days for a total of 13 sequential mating trials for each of the 25 males. During the first 3 mating trials, the females as well as the males drank the test solution at an average daily rate of 142 mg/kg body weight. After 3 trials, the mode of CHS administration was changed to gavage (220 mg/kg/day) so that the treatment was restricted to the males. Control males received distilled water by gavage. After another 4 mating trials, CHS treatment was suspended for the duration of 3 trials. During the final 2 breeding trials, CHS treatment by gavage (220 mg/kg/day) was re-instituted in the males.

Fifteen days after being separated from the males, the females were sacrificed to ascertain pregnancies, and to record the numbers of viable and nonviable embryos and resorption sites. The fetuses were examined for external defects and skeletal malformations.

Male fertility was calculated as the number of females impregnated relative to the number exposed. The male fertility and implantations (viable and nonviable embryos and resorption sites) data were analyzed using the Sign Test for time effects and the Wilcoxon–Mann–Whitney ranking test for treated:control comparisons[5].

Fertility in the treated group was generally impaired relative to the control group ($P < 0.05$ by one-tailed test). Fertility is plotted in Figure A. The adverse effect was apparently related to CHS dosing of males since the effect continued after treatment of females was halted. Observation on mating behaviour revealed no reduction in male or female libido. The antifertility effect persisted during the 3 trials in which the CHS treatment was suspended, indicating the effect to be of more than transitory nature.

The incidences of resorption sites and nonviable embryos in the test and control groups were similar in all trials, which excludes the possibility of a postimplantation embryocidal effect associated with CHS. However, the average number of implantations per litter was consistently and significantly ($P < 0.01$ by one-tailed test) decreased in the treated group. Data on viable and nonviable embryos and resorption sites were combined to compare postimplantational reproductive efficiency in both groups (Figure B). The depression in numbers of implantations could be accounted for by preimplantational loss apparently due to CHS treatment. In a previous study[3] in which the maximum dose of CHS was lower than that reported here, interference with embryonal viability at pre- and postimplantation stages was not observed; however, the antifertility effect of CHS in males was observed at as small a dose as 22.26 mg/kg/day as measured by ability to induce pregnancy.

Since the incidence and types of external defects and skeletal anomalies in embryos obtained from test animals were not different from the controls, the possibility

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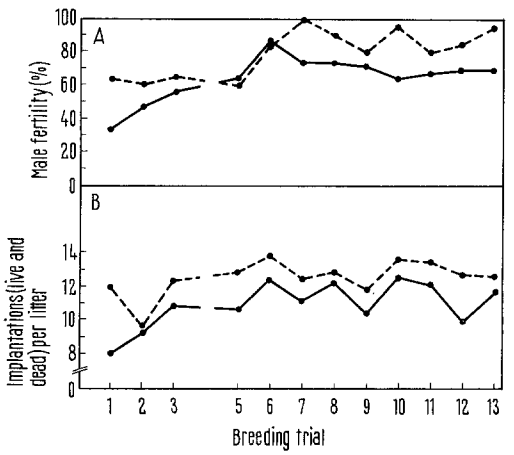
of a dominant mutation effecting the skeleton is remote. Resorption sites are considered a measure of dominant lethal mutation. When females are not treated, a decrease in litter size without a concomitant increase in resorption sites might be due to preimplantational wastage as a result of either decreased ability of the sperm to fertilize or low viability of the zygotes, prevention of mitotic activity, or inability of the blastocyst to implant. A previous cytological study of semen of CHS-treated rats recovered from the vagina after mating indicated no visible change in sperm morphology or motility. Further work is required to elucidate the nature of the decreased litter size and impaired fertility observed following CHS treatment of males[6].



Male fertility (A), and average implantations (live and dead)/litter (B) in CHS-treated (solid lines) and control (broken lines) rats. Data from mating trials, 1–3 (CHS-treated males and females), 5–8 and 12–13 (CHS-treated males only) and 9–11 (neither sex treated) is plotted.

Résumé. Des rats mâles traités oralement avec du sulfate de cyclohexylamine (220 mg/kg/jour) ont été accouplés à des femelles traitées ou non avec du sulfate de cyclohexylamine. Dans les 2 cas, il y a eu des descendants moins nombreux que chez les contrôles. Les résultats suggèrent que l'effet est transmis par les mâles traités et est, peut-être, causé par un dommage génétique exprimé avant l'implantation de l'embryon.

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[6] Acknowledgements. The authors are thankful to Mr. D. A. LYON for assistance in analyzing the data and to Mrs. L. L. WHITTA for technical assistance.

11β-Methyl-19-Norsteroids: Novel Progestational Hormones

A continued search for substances which may have advantages over known oral contraceptives had led us to the discovery that in experimental animals certain 11β-methyl-19-norsteroids, as single substances, possess some of the hormonal properties characteristic of mixtures of steroid hormones presently used in human fertility control[1] and lack other undesirable properties. The synthesis and some of the biological properties of 17α-ethynyl-17β-hydroxy-11β-methylestr-4-en-3-one (XII) and 3β,17β-diacetoxy-17α-ethynyl-11β-methylestr-4-ene (XIII) are described presently.

Thus the readily available 17,17-ethylenedioxyestra-1,3,5(10)-triene-3,11β-diol[2] (I) was converted with methyl iodide, methanol, and potassium carbonate to II[3], mp 127–128°, which was oxidized with 8*N* chromic-sulfuric acid in acetone at 0° to the ketone III. Treatment of the crude ketone III with methyl magnesium bromide followed by hydrolysis of the product IV in strong acid yielded 11β-hydroxy-3-methoxy-11α-methylestra 1,3,5,(10)-trien-17-one (V), mp 178–179°, λ_{max} 2.72, 5.71, and 6.18 μ, NMR maxima at 67 and 99 (C-11 and C-13 methyls) Hz. Dehydration of V in refluxing benzene containing *p*-toluenesulfonic acid provided 3-methoxy-11-methylestra-1,3,5(10), 9(11)-tetraen-17-one (VI), mp 95° λ_{max} 257.5 nm (ε = 18,050) which upon hydrogenation in methanol with Pd-C afforded 3-methoxy-11β-methylestra-1,3,5(10)-trien-17-one (VII), mp 152°, NMR maxima at 51 and 58 (C-11β methyl) and 62 (C-13 methyl) Hz and, in minor amount, 3-methoxy-11α-methyl-9β-estra-1,3,5(10)-trien-17-one (VIII), mp 129–130°, NMR maxima at 75 and 83 (C-11α methyl) and 61 (C-13 methyl) Hz. Reduction of VII with sodium borohydride gave the corresponding alcohol IX, mp 108–110°, which upon reduction with sodium, ammonia, and *t*-butyl alcohol followed by Oppenauer oxidation of the product with aluminum *i*-propoxide, cyclohexanone, and refluxing toluene yielded the ketone X, mp 140–142°. Ethynylation of X with lithium acetylide in tetrahydrofuran followed by hydrolysis of the product XI in strong acid yielded 17α-ethynyl-17β-hydroxy-11β-methylestr-4-en-3-one (XII), mp 222–223°, λ_{max}^{MeOH} 241 nm (ε = 17,500), ν_{max}^{KBr} 2.92, 3.07, and 6.00 μ. Reduction of XII with lithium tri-*t*-butoxyaluminum hydride followed by diacetylation of the product afforded 3β,17β,diacetoxy-17α-ethynyl-11β-methylestr-4-ene (XIII), mp 148–150°.

A buffered hormonal action is characteristic of XII and XIII. In experimental animals, they exhibit potent progestational activities, anti-estrogenic activities, and estrogenic responses in the estrogen deficient state.

Thus, in the Clauberg assay[4] for progestational activity the activities of XII, XIII, and XIV when administered s.c. were 25:25:1, respectively, and when administered orally were 10:10:1, respectively[5]. In the rat vaginal

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[2] J. S. BARAN, J. med. Chem. *10*, 1188 (1967).

[3] This substance and others prepared below gave satisfactory analyses. The NMR-spectra were determined in deuterochloroform on a Varian Model A-60 spectrometer at 60 Mc with Me_4Si as an internal standard.

[4] C. W. EMMENS, in *Hormone Assay* (Academic Press, Inc., New York, N.Y. 1950), p. 422.

[5] XIII also exhibits potent anti-estrogenic activity in the immature female mouse treated with estrogen which correlates well with the progestational activity obtained in the Clauberg assay.