Self-administered nicotine solutions preferred to placebo by the rat

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Nicotine solutions were self-administered by male, hooded Lister rats (180–250 g) either by drinking water or through permanently implanted catheters.

Rats were trained to obtain their total water requirement in the home cage by pressing levers. Two wells provided water, and the rats, kept either singly or in groups of four, usually drank more from one well than from the other. Nicotine solution (nicotine acid tartrate, 50 μg/ml.) then replaced water in one of the wells. Use of that well increased in each of the grouped rats (four groups) and in three of five of the rats kept singly. This was also true if nicotine replaced water in the least preferred well.

Polyethylene catheters implanted in the external jugular veins of thirty-two naïve rats were permanently connected to an automatic injection apparatus. A programmed sequence of nicotine injections was given for at least a week to the rats in their home cages. After this time the programme was switched off and rats had to learn to press a lever to obtain nicotine. Twelve rats survived with patent catheters and all of these learned to press the lever. The rate of lever-pressing increased by more than one-fifth in six rats when the dose of nicotine received for each press was reduced from 10 to 5 μg/kg. In a separate experiment in which rats could obtain the saline vehicle by lever pressing, no rat opted to obtain this reward.

Twelve rats were trained to press a lever for water rewards in daily 1 hr trials in special cages. Once response rates had become steady polyethylene catheters were implanted as before. In subsequent trials, a lever-press also injected saline simultaneously with the water reward. The water reward was now removed.

Half of the rats still received a saline injection and the other half now received a nicotine injection (1 μg per press). Saline rats extinguished rapidly. The number of lever presses of the nicotine rats fell at first and then rapidly increased back towards the original pressing rate. The consumption of nicotine was about 50 μg in each trial. These rats preferred nicotine solutions to a placebo of either water, in one test, or saline in the other.

Man uses tobacco in diverse ways; these experiments support suggestions that nicotine may be responsible for some of its appeal.

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The effect of progesterone and some other agents on the failure of pregnancy produced by feeding agroclavine, an ergot alkaloid, in the rat

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Failure of pregnancy occurs in mice after oral administration of agroclavine during the first five days after mating (Mantle, 1969) and this observation has been extended to rats (Edwardson, 1968). In an attempt to elucidate the mechanisms involved in this failure, prolactin, progesterone and other agents have been administered by injection during the period of treatment with agroclavine.

Female rats were mated and the date of mating, day 1 of pregnancy, determined by the presence of sperm in the vaginal smear. Agroclavine was dissolved in a 3% tartaric acid solution and added to ground pellet diet to give a concentration of alkaloid 10 mg/100 g diet. The diet was given *ad lib.* from day 2 to day 6 of pregnancy and the mean daily food intake over this period of 18 g per rat did not differ significantly from control values.

Daily intraperitoneal injections of ovine prolactin (50 i.u.), progesterone (2 mg), progesterone (4 mg) with oestradiol benzoate (1 μg), chlorpromazine (5 mg) and reserpine (25–100 μg) were given to different groups of rats on the agroclavine diet from day 2 to day 6. The effect of these agents on pregnancy was determined by macroscopic and histological examination of the uteri on day 15, and the results are shown in Table 1. There was no evidence that implantation had occurred in the rats which failed to produce litters at term.

TABLE 1. *Effect of progesterone and some other agents on the failure of pregnancy produced by feeding agroclavine in the rat*

Group	Treatment	Effect on pregnancy			
		No. of rats mated	No. of rats pregnant	% pregnant	Mean No. of foetuses /litter
1	Control diet	35	31	89	10·5
2	Agroclavine diet	32	0	0	0
3	Agroclavine diet+ prolactin (50 i.u.)	11	5	45	9·6
4	Agroclavine diet + progesterone (2 mg)	11	9	82	3·6
5	Agroclavine diet+ progesterone (4 mg)+ oestradiol (1 μg)	4	1	25	1·0
6	Agroclavine diet+ chlorpromazine (5 mg)	5	5	100	5·8
7	Agroclavine diet+ reserpine (25–100 μg)	5	4	80	6·2

Agroclavine was administered in the diet from day 2 to day 6 of pregnancy; the mean dose per rat per day was 1·8 mg. Other substances were given by daily intraperitoneal injection over the same period.

Further groups receiving the treatments described were allowed to proceed to term. Prolactin and progesterone-treated animals produced litters on the 21 and 22 days after conception while the chlorpromazine and reserpine treated rats littered on days 25 to 27, indicating that a delay of implantation had occurred in these latter groups.

The possibility that agroclavine interferes directly with the action of progesterone on the uterus was tested in groups of rats ovariectomized on day 12 of pregnancy and given daily injections of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg of progesterone while maintained on the agroclavine diet. Agroclavine had no significant effect on the number of foetuses maintained alive until day 20 at different dose levels of progesterone when compared with ovariectomized, progesterone-treated rats on the control diet.

These results suggest that agroclavine may be acting either directly on the ovaries or through the hypothalamo-hypophysial system to produce a disturbance of endocrine functions.

Agroclavine was prepared by Dr. P. G. Mantle, Department of Biochemistry, Imperial College, London.

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The antagonism of 17β-oestradiol by stilboestrols methylated in the phenyl rings

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Clark & O'Donnell (1965) found that the presence of four methyl substituents situated *ortho* to the phenolic hydroxyl groups in ψ-diethylstilboestrol produced an anti-oestrogen which was almost devoid of oestrogenic activity. Among related compounds lacking methyl groups in the nucleus, Emmens, Cox & Martin (1958, 1959) reported optimal anti-oestrogenic potency in dimethylstilboestrol and butoestrol. The effects of chain length in nuclear methylated derivatives of stilboestrol have been investigated by using 3,3′,5,5′-tetramethyl-α,β-diethylstilboestrol (I), 3,3′,5,5′-tetramethyl-α,β-dimethylstilboestrol (II) and 3,3′,5,5′-tetramethylstilboestrol (III).

In the uterine weight test in immature mice, only compound I was oestrogenic, being 2.4×10^{-4} times as potent as 17β-oestradiol [fiducial limits ($P=0.95$) 1.4×10^{-4}–3.2×10^{-4}]. Subcutaneous administration of mixtures of compound I (0.02 mg) or compound II (1 mg) with 17β-oestradiol (0.03–0.06 μg) in arachis oil failed to suppress the response of the uterus to oestradiol, but intra-vaginal administration to ovariectomized mice of mixtures of test compound I or II (5 μg) with 17β-oestradiol (1.5×10^{-4} μg to 13.5×10^{-4} μg) in 2% aqueous Tween 80 (0.01 ml.) demonstrated a highly significant inhibition of the vaginal cornification produced by oestradiol. Compound III was inactive in this latter test.

In order to elucidate further the mechanism of this oestrogen antagonism, the effect of compounds I and II on the uptake of tritiated oestradiol was investigated. The test compound (2.5 μg), was administered intravaginally together with 7.5×10^{-5} μg of [6,7-^{3}H]oestradiol-17β in 0.0025 ml. 2% aqueous Tween 80. Both I and II exhibited highly significant blockade of the uptake of the tritiated oestradiol by the vagina. Preliminary experiments on homogenized vaginal tissue followed by subcellular fractionation indicate that the main site of uptake of the tritiated oestradiol is the "nuclear-myofibrillar" fraction and that uptake by this fraction is inhibited by compound I.

Aqueous Tween 80 (2%) is believed to be a suitable vehicle because intravaginal administration of 0.01 ml. of a 10% w/v solution of Tween 80 containing 17β-oestradiol (0.4×10^{-4} and 0.8×10^{-4} μg) did not produce a significant difference in the mitotic index or epithelium thickness from that obtained with aqueous solutions of oestradiol. Furthermore intravaginal administration of 0.01 ml. of aqueous Tween 80 alone (concentration up to 40% w/v) did not alter the normal microscopic appearance of the vaginal epithelium.

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