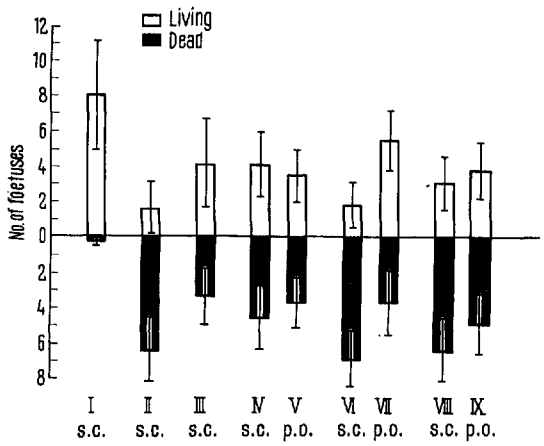
when administered parenterally. However, no further increase in activity was found with a 5–10-fold s.c. dosis of Cl-DMAP (columns VI and VIII). In contrast to the failure of maintaining pregnancy with various p.o. doses of DMAP in pregnant spayed rats (unpublished data) the oral administration of chlor derivative had a marked effect. The results of groups V, VII and IX (i.e. 0.1 mg, 0.5 mg and 1.0 mg of Cl-DMAP) suggest for a balanced ratio between the average number of living and dead foetuses, the dose of 0.5 mg being in this respect the most advantageous one. Also in this experiment a further increase in dose did not enhance the effect.

Discussion. The present experiments were based on a previous unpublished observation which suggests that with the exception of extremely high doses of progesterone the above-mentioned gestagens alone are incapable of maintaining pregnancy in spayed rats without simultaneous administration of corresponding doses of estrogen. A similar observation was made by other authors too[3]. By evaluation of the above-mentioned results, a good relation between the administered doses and the pharmacological response was obtained. A comparable effect was produced by 1.0 mg of DMAP and 0.1 mg of Cl-DMAP given parenterally, the effect of 1.0 mg of DMAP being more pronounced than that of 10 mg of progesterone. These results parallel very closely with the values of progestational activity obtained by the modified McPHAIL test according to which DMAP and Cl-DMAP were shown in repeated experiments to be 10–13 and 75 times as effective as progesterone[4]. Interestingly enough, the chloro derivative maintained the pregnancy when administered both s.c. and orally, whereas the parent steroid (DMAP) was effective only by parenteral route. However, the different response to DMAP in s.c. and p.o. administration has been observed. Whereas in infantile female rabbit the substance was effective in both routes, only the s.c. administration was shown to be effective in rat. This difference might be related to a possible different metabolic utilization of this substances by both species. Within this group of substances under study there was shown a general parallelism between both types of assay used.



Mean values of living and dead foetuses per 1 female. Column: I Non-spayed females, II progesterone 10.0, III DMAP 1.0, IV Cl-DMAP 0.1, V Cl-DMAP 0.1, VI Cl-DMAP 0.5, VII Cl-DMAP 0.5, VIII Cl-DMAP 1.0, IX Cl-DMAP 1.0 mg female/day. Significance of difference of living foetuses: II–IX; II–VII. Significance of difference of dead foetuses: II–III; II–V; II–VII; VI–VII, No. of living and dead foetuses in column I is significantly different from all other columns.

Accordingly it seems advisable to use the test for maintenance of pregnancy as a standard supplementary test to the classical McPHAIL modification of CLAUBERG assay when characterizing general progestational activity of a new synthetic gestagen. This measure is even more desirable in the light of the results of TALWALKER et al.[2] who observed discrepancies between both methods of assaying within another series of substances under test.

Zusammenfassung. DMAP 1 mg oder Cl-DMAP 0,1 mg pro Tag zeigten eine mit der von 10 mg Progesteron vergleichbare schwangerschaftserhaltende Wirkung. DMAP war nur s.c., Cl-DMAP auch p.o. wirksam. Gleichzeitige Verabfolgung von Ethinylöstradiol war unentbehrlich. Es zeigte sich eine Parallelität mit dem McPhail-Test.

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Sex Hormones and Concentration of Noradrenalin and Dopamine in the Anterior Hypothalamus of Castrated Rats[1]

Castration of adult male and female rats produces an increase of noradrenalin[2,3] and decrease of dopamine[3] in the anterior hypothalamus. No changes were detected in the other zones of hypothalamus or in the cerebral cortex after gonadectomy. The changes of catecholamines in the hypothalamus after castration are simultaneous[3] with the marked increase of gonadotrophins produced under these conditions[4,5], suggesting that these amines might be involved in the control of gonadotrophin secretion of the pituitary gland.

It was assumed that the modification of hypothalamic noradrenalin and dopamine was caused by removal of the influence of sex hormones upon the hypothalamo-hypophysial axis. This work was dedicated to evaluate the effects of gonadal steroids on the catecholamine levels in the anterior hypothalamus of ovariectomized rats.

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Methods. Castrated female rats weighing 150–180 g were used. The animals were housed in an air conditioned room at 22 ± 1 °C and 14 h of light daily, and sacrificed in the afternoon. Noradrenalin and dopamine were assayed in pools of hypothalami taken from 6 rats each. The anterior hypothalamus extended from the anterior border of the optic chiasma to 1 mm behind its caudal border[2]. The pooled pieces (approximate weight 45 mg) were homogenized in 5 ml of cold 0.4 N perchloric acid, and catecholamines were determined fluorimetrically[6–8].

The hormones used were: 17-β-estradiol benzoate (Syntex) and progesterone (Laboratorios Gador, Schering). They were injected s.c. in 0.2 ml of corn oil (Mazola), controls were injected with oil.

Results. The Table shows the results obtained. The values of catecholamines in the ovariectomized untreated rats were similar to those previously reported[2].

Effects of estradiol benzoate. Estradiol benzoate was injected daily at a dose of 0.4 µg per rat, starting on the day of operation and going on for 14 days. This did not prevent the increase of noradrenalin and decrease of dopamine in the anterior hypothalamus observed in castrated animals. In other experiments, similar results were obtained with 2 µg of estradiol, injected daily during 20 days.

Combined administration of estradiol and progesterone. 2 different schedules of treatment were employed in these series. First, (a) the effects of low doses of each hormone were evaluated. In the other experiments (b) high doses were used.

(a) Daily treatment of castrated rats with 5 µg of estradiol benzoate and 2 mg of progesterone failed to modify the levels of noradrenalin in the anterior hypothalamus as it is shown in the Table (this treatment was begun on the 20th day after ovariectomy and was applied daily for 1 week). At the same time the weight of the anterior pituitary was significantly increased ($P < 0.001$) by the hormones. In the treated rats the weight of the gland was 13.4 ± 0.7 mg, and in the controls it was 8.8 ± 0.4 mg.

Effects of gonadal hormones on the content of noradrenalin and dopamine in the anterior hypothalamus of ovariectomized rats

Catecholamine content in µg/g of tissue

Treatment	Daily dose	No. of pools	Noradrenalin ± S.E.[a]	Dopamin ± S.E.[a]
Controls	–	14	2.06 ± 0.16	0.30 ± 0.01
Estradiol benzoate	0.4 µg	8	2.39 ± 0.13	0.30 ± 0.01
Estradiol benzoate	2 µg	2	1.83 ± 0.09	–
Controls	–	4	2.01 ± 0.19	–
Estradiol + progesterone	5 µg 2 mg	5	1.74 ± 0.10	–
Controls	–	6	2.01 ± 0.13	0.30 ± 0.005
Estradiol + progesterone	50 µg 25 mg	8	1.57 ± 0.04[b]	0.44 ± 0.01[c]
Estradiol	50 µg	4	1.77 ± 0.14	–
Progesterone	25 mg	2	1.84 ± 0.20	–

[a] S.E., standard error of the mean, [b] $P < 0.01$ when compared with control value, [c] $P < 0.001$.

(b) Employing higher doses of estradiol-progesterone, 50 µg and 25 mg respectively, administered for 2 days in rats which had been castrated 20 days before and sacrificing the animals on the next day, a decrease of noradrenalin and increase of dopamine in the anterior hypothalamus was observed. The separate injection of estradiol or progesterone in other groups of rats were ineffective.

Discussion. The action of sex steroids on the catecholamine content of the rat hypothalamus was analysed in the present work using ovariectomized rats. As it was found, treatments with low doses of estradiol were ineffective to prevent the changes or to reduce the level of noradrenalin, increased by castration, in the anterior hypothalamus. Similarly, no effects of the combined estradiol-progesterone treatment were observed when applied at the dose of 5 µg and 2 mg respectively, although this treatment influenced the weight of the anterior pituitary as it has been classically described. These doses also inhibit the release of gonadotrophins in castrated rats[5]. When large doses of estradiol plus progesterone were employed, noradrenalin was restored at the levels found in normal rats during estrus[2,9]. These results indicate that the action of gonadal hormones on the hypothalamic catecholamines occurs at a higher dose level than the one which is necessary for its inhibitory effects on the hypophysis.

Similarly, as it was found for catecholamines, the content of hypothalamic neurohumours (releasing factors) in the median eminence seems to be reduced only by large doses of sex hormones. Injecting high doses of estradiol, the content of FSH-releasing factor is lowered in castrated female rats[10]. The LH-releasing factor is reduced by testosterone employed in large doses[11].

Our report shows that the increase of noradrenalin and decrease of dopamine in the anterior part of hypothalamus produced by castration could be in part due to the reduction of circulating sex hormones[12].

Zusammenfassung. Die Autoren stellen fest, dass bei Ratten durch Kastration der Gehalt an Noradrenalin und Dopamin im vordern Teil des Hypothalamus ansteigt. Erst hohe Dosen von Östradiol und Progesteron können den Kastrationseffekt wieder zum Verschwinden bringen.

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