It is of interest that Mitropoulos and Myant[5] in similar experiments observed none or only a small thyroxine effect on mitochondrial hydroxylation of the cholesterol steroid nucleus, including 7α-hydroxylation. This difference may be explained by a different way of 7α-hydroxylation of DHA and cholesterol, respectively e.g. by the absence of non-enzymatic 7-hydroxylation of DHA which is pronounced in the case of cholesterol in vitro by a different affinity of steroid hydroxylase towards the 2 substrates which would lead to a different susceptibility of both reactions. The observed decrease in hydroxylation ability of liver after thyroxine is possibly connected with the autooxydation effect of thyroxine as described by Bunyan et al.[6] and Kaufmann et al.[7]. A lower extent of formation of lipid oxydation products in rats after thyroxine application has also been observed by Lejsek and Šimek[8]. These authors also investigated the effect of the diet on lipid oxydation and have found somewhat higher values in undernourished rats as compared with rats kept on a normal diet. This may be associated with our observation of enhanced hydroxylation in fasting rats. The problem of steroid metabolism in fasting is more complex and would have to be studied in greater detail.

Effect of thyroxine application and fasting on 7α-hydroxylation of dehydroepiandrosterone in rat liver homogenate

Duration of fasting	μg 7α-OH-DHA in incubation sample (average ± S.D.)	
	Control animals	Thyroxine treated animals
0 h	11.45 ± 1.47	4.62 ± 1.23
12 h	12.58 ± 10.85	6.59 ± 3.51
36 h	33.30 ± 19.78	14.22 ± 3.25
48 h	8.23 ± 4.00	9.10 ± 4.50

Zusammenfassung. Nach einer peroralen Thyroxingabe an weibliche Ratten wurde die 7α-Hydroxylierung von Dehydroepiandrosteron in vitro in den Leberhomogenaten der satten sowie der hungrigen Tiere vermindert. Durch eine 36 h Hungerperiode wurde die 7α-Hydroxylierung von DHA bei Kontrollen und ebenso bei thyroxinbehandelten Ratten signifikant erhöht.

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Maintenance of Pregnancy in Spayed Female Rats by Gestagens of 6-Dehydro-16-Methylene-17α-Acetoxyprogesterone Type

In the present series of experiments with substituted gestagens of 6-dehydro-16-methylene-17α-acetoxyprogesterone type, the principal intention was to study the relations between the modified McPhail test[1] and a test evaluating the effect of gestagens on the maintenance of pregnancy in spayed female rats. The latter shows the potency of gestagen as a substitute for endogenous progesterone. A further purpose of this study was to verify published data on apparent discrepancies between the Clauberg assay and the latter one with several progestational substances[2].

Material and methods. Secundiparous rats were housed in cages with fertile males. At least 10 females formed each experimental group. The day on which spermatozoa were found in the vaginal smear was considered as the first day of the experiment. On the 8th day the administration of gestagens, i.e. 6-dehydro-16-methylene-17α-acetoxyprogesterone (DMAP) and of 6-Cl-6-dehydro-16-methylene-17α-acetoxyprogesterone (Cl-DMAP) in 0.4 ml of olive oil was started simultaneously with 0.4 μ of ethinylestradiol in 0.2 ml of olive oil. On the 9th day the females were spayed. 24 h following the last administration, the females were sacrificed by cervical dislocation, the uteri removed immediately, dissected and the number of living and dead foetuses estimated.

Progesterone was administered s.c. in a dose of 10 mg; DMAP and Cl-DMAP were given both s.c. and orally at daily doses of 0.1, 0.5 and 1.0 mg per animal. DMAP given orally was ineffective even at high dose levels (unpublished). The results were statistically evaluated by the *t*-test.

The results are summarized in the Figure which indicates the relation between the mean values of living (white columns) and dead (black columns) foetuses found after hysterectomy and expressed as average for 1 female of each group.

The Figure indicates a marked activity of synthetic gestagens in substituting endogenous progesterone. Whereas in the unoperated control group (column I) a high average number of living foetuses and a small number of resorptions was found, the parenterally administered progesterone (column II) in a dose of 10 mg maintained a relatively small number of living foetuses as compared with a high number of dead ones. In the group treated s.c. with DMAP (column III) in a dose of $^1/_{10}$ of that of progesterone, more than 50% of living foetuses was found. A 0.1 mg dose of the chlorinated derivative, i.e. $^1/_{100}$ of that of progesterone produced a comparable effect

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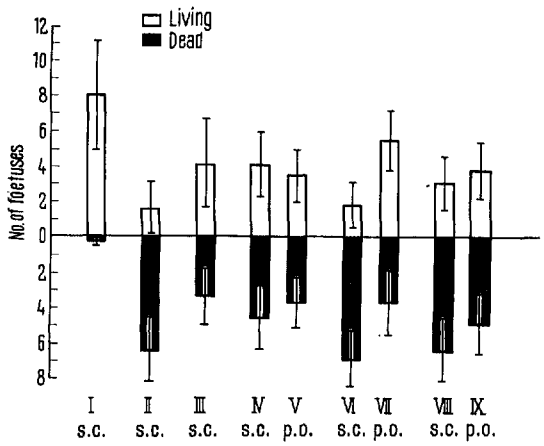
when administered parenterally. However, no further increase in activity was found with a 5–10-fold s.c. dosis of Cl-DMAP (columns VI and VIII). In contrast to the failure of maintaining pregnancy with various p.o. doses of DMAP in pregnant spayed rats (unpublished data) the oral administration of chlor 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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