Riteniamo opportuno precisare che nelle varie fasi della tecnica esposta, abbiamo preferito adoperare, come diluente, lo stesso liquido plasmatico del tumore, via via ottenuto con le varie centrifugazioni, allo scopo di eliminare, ogni shock derivante dal cambiamento del veicolo liquido. Tale precauzione ci è parsa tanto più opportuna, in quanto sconosciamo le caratteristiche biologiche, e particolarmente quelle di resistenza, del presunto agente etiologico.

In una prima fase dell'esperienza sono stati inoculati, con ml 0,2 ciascuno di tale estratto tumorale n. 7 rattini neonati di circa 36 h, e con ml 1 ciascuno n. 4 ratti femmine adulte. Le inoculazioni sono state, in ogni caso, effettuate in sede peritoneale, nella regione ombelicale sinistra.

In una seconda fase sono stati invece inoculati, con le medesime modalità, n. 10 rattini neonati di 14 h e n. 2 ratti maschi adulti.

A distanza di qualche giorno sono deceduti, per ragioni estranee all'esperienza, 2 rattini del primo gruppo di soggetti e 3 del secondo gruppo.

Dei 12 rattini neonati sopravvissuti in complesso, 1 ha contratto il tumore di Yoshida solo in forma solida, e 10 sia in forma ascitica che solida. Quest'ultima, costituita da nodi e noduli tumorali, trovava sede nel fegato, nel pancreas, nel diaframma e, soprattutto, nel parenchima polmonare. La neoplasia si è resa clinicamente manifesta in un periodo di tempo compreso fra 16 e 30 giorni dall'inoculo.

Dei 6 ratti adulti inoculati, soltanto in 1 si è sviluppata la forma ascitica del tumore.

La natura neoplastica delle lesioni ottenute è stata sempre accertata con l'esame istologico o citologico secondo la forma del tumore sviluppatosi.

Il liquido ascitico ottenuto dai rattini neonati, si è dimostrato a sua volta perfettamente idoneo a determinare la consueta neoplasia ascitica nei ratti adulti[3].

Summary. The authors demonstrate the transmissibility of the tumour of Yoshida by means of definitely acellular, tumoural extracts. Neoplasia occurred in 11 out of 12 new-born mice i.p. inoculated as above. It is noteworthy that the neoplasia developed not only ascitic but also solid (nodes and tumoural nodules) prevalently at pulmonar level. Derived tumoural ascites are transmissible to the adult rat.

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The Effect of Thyroid Application and Fasting on 7α-Hydroxylation of Dehydroepiandrosterone in Rat Liver in vitro

The effect of thyroid hormone on steroidogenesis and steroid hormone metabolism has been thoroughly studied in human and experimental animals. Thus Jao and Koritz[1], for instance, have found an inhibiting influence of thyroxine on 2 stages of corticoid biosynthesis from cholesterol, but not on 11β-hydroxylation. In oestrogen catabolism thyroxine increases 2-methoxylation and 2-hydroxylation, since C-2 and C-16 hydroxylation of oestrone are competitive reactions which are inhibited by thyroxine in different ways[2]. The activity of steroid Δ^4-5α-reductase in rat liver microsomes is enhanced by thyroxine administration as well[3].

It was of interest to ascertain whether the enzyme system hydroxylating dehydroepiandrosterone (Δ^5-androsten-3β-ol-17-on, DHA) in the 7α-position in rat liver homogenates, would be affected by thyroxine and possibly also by keeping the animals in the fasting state.

The rats used (Wistar females, weight 160–200 g) were divided into 2 groups; one was fed by a standard diet (controls) and the diet of the other group was augmented by a daily dose of 4.0 mg L-thyroxine per animal for a duration of 7 days. Food intake was recorded. Then 8 animals of the control group and 8 thyroxine treated animals were sacrificed by decapitation, 0, 12, 36 and 48 h respectively after cessation of food intake (water was allowed ad libitum). To 10 ml of homogenate prepared from 1 g liver in Ringer-Krebs phosphate buffer (pH 7.4) was added 0.12 ml of 5% glucose solution and 2 mg DHA in 1.0 ml triethylene glycol and after saturation with oxygen incubation at 38 °C was maintained under constant agitation for 60 min. The incubation mixture was then extracted with ethyl acetate, the extract washed with saturated sodium bicarbonate solution and water and after evaporation to dryness chromatographed on a thin layer of alumina and then rechromatographed on paper in Bush B5 system as previously described[4]. Estimation of 7α-OH-DHA was performed densitometrically.

The results of 7α-OH-DHA estimation in the individual groups of animals are presented in the Table. It is apparent that thyroxine application suppressed 7α-hydroxylation in liver. The lowering is statistically significant (significance level 0.05) and it may be observed both in normally fed rats as well as in rats after 12 and 36 h fasting. The effect of fasting appears only 36 h after cessation of food intake; at this stage there is a threefold content of 7α-OH-DHA (as compared to zero time) in the incubated samples both in the controls as well as in the thyroxine treated animals (the increase is also statistically significant at the 0.05 level). After a further 12 h fasting, however, there again appears a decrease, which is particularly pronounced in the control animals.

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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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