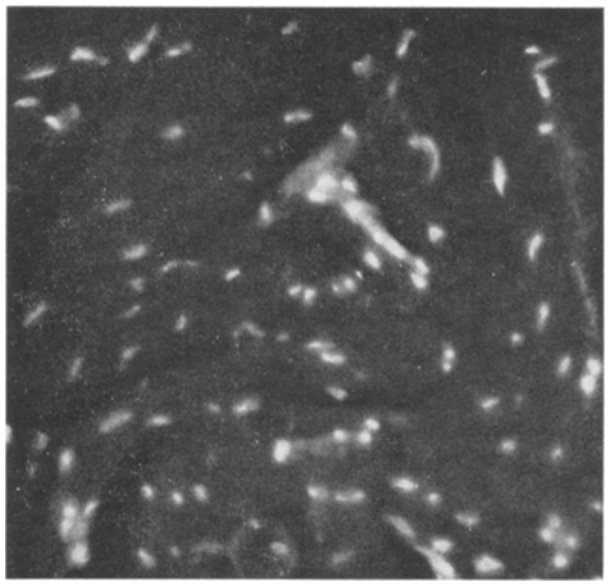

Fig. 3. Keloid. Large number of histamine-containing mast cells are visible after treatment with OPT. × 200.

With the technique used, mast cells were the only tissue component in the skin exhibiting a fluorescence upon OPT treatment. The content of mast cells in the various skin regions was very well correlated with the concentration of histamine as measured chemically[14].

Zusammenfassung. Die Haut von Patienten mit Urticaria pigmentosa und Keloiden wurde mit der histochemischen *o*-Phthaldialdehyd-Technik untersucht. Die Lokalisation von Histamin konnte durch Fluoreszenzmikroskopie festgestellt werden.

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The Effect of Monoamine Synthesis-Inhibitors on the Ovarian Compensatory Hypertrophy

There is growing evidence that hypothalamic catecholamines[1,2], and thus probably serotonin[3], are involved in the neuroendocrine mechanisms that control the secretion of pituitary gonadotrophins. It has been established that drugs modifying the rate of synthesis of catecholamines block ovulation[4,5] and induce pseudopregnancy in rats[5]. According to COPPOLA[6] the brain but not the peripheral catecholamines are responsible for these effects.

In this paper we report experiments showing the effect on the ovarian compensatory hypertrophy of 2 drugs that inhibit the synthesis of brain monoamines. L-α-methyl *p*-tyrosine (MPT) an inhibitor of catecholamine synthesis[7] and L-*p*-chlorphenylalanine (ClPh) that blocks synthesis of serotonin, were used[8]. Experimental and control Holtzman rats were unilaterally ovariectomized concomitant with the first injection. The other ovary was removed 10 days later. The ovarian compensatory hypertrophy (OCH) was evaluated by comparison of the relative weights (absolute weight/g of body weight) of both ovaries. In addition, ovaries were histologically studied in paraffin sections with the Masson thrichromic staining. Vaginal smears were obtained daily in controls and experimentals. Mammary glands were removed at autopsy and analyzed in sections stained with hematoxilin-eosin.

Results. (1) α-methyl *p*-tyrosine (MPT): A continuous i.p. administration of this drug at daily doses of 150 mg/kg[9] completely suppressed the OCH. In the controls, the ovaries which were studied simultaneously, showed a 59% hypertrophy (Table). Figures 1–4 show the histological characteristics of the ovaries. Normal hypertrophied ovaries are characterized in 5 μ sections by the presence of 5–7 large and active corpora lutea interspersed with mature follicles, some of them quite large. On the contrary, sections of ovaries of the group treated with MPT showed just 1 or 2 large corpora lutea and abundant mature follicles. The stroma appeared more densely populated with interstitial cells than in the control ovaries. Vaginal smears, on the other hand, showed for controls a normal estrous cycle whereas the experimental rats presented a constant diestrus. The mammary gland showed the characteristic lobulo-alveolar development reported for rat pseudopregnancy[10]. Treatment with the catecholamine inhibitor reduced the increase of body weight with respect to the controls which showed a 15 g gain in weight. Therefore, no weight losses, as reported after reserpine treatment, which also blocks OCH[11], were found.

(2) Combined administration of α-methyl *p*-tyrosine and DOPA: MPT was injected as described above. Simultaneous treatment with L-DOPA (dihydroxyphenylalanine) at daily doses of 100 mg/kg prevented the blocking effects of MPT on the OCH. No statistical differences were found between the weight of the ovaries of the MPT plus DOPA treated rats and those of the controls. Ovaries, as shown in Figure 3, presented several growing follicles, and 5–7 large corpora lutea. At the stroma, interstitial cells were found in numbers similar to the controls.

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(3) *p*-Chlorphenylalanine: Ovarian compensatory hypertrophy was not modified by a daily treatment with this enzymatic inhibitor of 5-hydroxy-tryptamine synthesis at doses of 250 mg/kg, which are known to be effective to deplete serotonin[8]. According to our observations, this monoamine seems not to be involved in the ovary response.

Discussion. The mechanisms of both the ovarian compensatory hypertrophy and their blockage after catecholamine depletion are not clearly explained. OCH is generally assumed to result from an increased secretion of gonadotrophins following unilateral ovariectomy. Higher serum levels and decrease of pituitary FSH in hemispayed rats were recently reported[12]. Earlier, other authors failed to detect any changes[13,14].

A significant finding is the higher number of corpora lutea produced after multiple ovulation which apparently explains the increase of ovary weight involved in compensatory hypertrophy. Our results suggest that the gonadotrophin output, causing the postovulatory luteinization, is prevented during the administration of MPT whereas at the same time there is a low level secretion of LH and FSH. In support of the former view we found, in a subsidiary experiment, that ovulation occurred after 48 h of the treatment-interruption at the 10th day. Some animals of this group were sacrificed 10 days after stopping treatment. In this case a higher degree of compensatory hypertrophy (+97 to +146% versus +70 to +127% in controls) was observed. At autopsy the ovaries showed 11–12 corpora lutea. The latter findings suggest that MPT treatment does not affect gonadotrophin synthesis, though it impairs secretion by affecting somewhat the releasing mechanisms.

It is also possible that the blockage of OCH obtained by the catecholamine inhibitor might be due to the pseudopregnancy effect of continued administration of MPT[4]. Pseudopregnancy in rats, as is known, prevents the hypertrophy of the remaining ovary following unilateral ovariectomy[15].

The results of our paper give further data on the role of catecholamines on gonadal function. The general

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Effects of enzymatic inhibitors of catecholamine and serotonin synthesis on rat ovarian compensatory hypertrophy (OCH)

Group	Body weight (g) Initial	Final	Ovary weight (mg/100 g) 1st	2nd	OCH %
Controls (15)	190 ± 3.3[a]	204.6 ± 2.7[b]	13.8 ± 0.8	22.0 ± 0.9	59.4
α-methyl *p*-tyrosine (11)	189 ± 4.1	190.0 ± 8.4	14.2 ± 0.8	13.6 ± 1.0[c]	0
α-methyl *p*-tyrosine + DOPA (5)	162.8 ± 4.1	177.8 ± 5.1[b]	16.0 ± 1.8	20.7 ± 1.7	29.3
p-Chlorphenylalanine (5)	165.1 ± 10.5	163.6 ± 8.1	16.0 ± 0.8	27.2 ± 3.1	70.0

[a] Mean ± S.E. of the mean. [b] $P < 0.01$; final vs. initial body weight. [c] $P < 0.01$, versus 2nd ovary of control; number of animals per group in brackets.

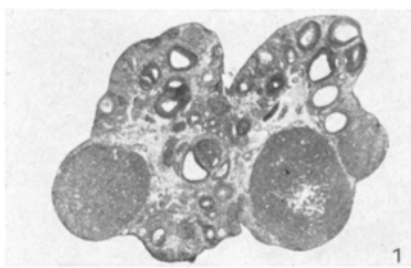

Fig. 1. Control ovary. Follicles at different stages of development and 3 corpora lutea are shown. × 15.

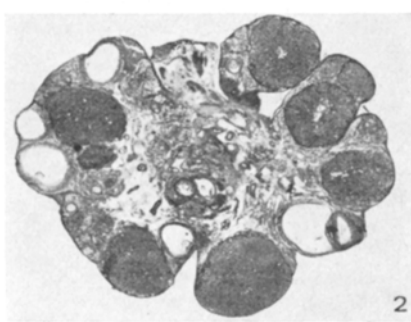

Fig. 2. Ovarian compensatory hypertrophy. The number of corpora lutea has been increased, and the interstitial tissue is well developed. There are also some large follicles. × 15.

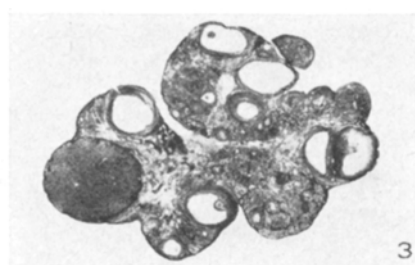

Fig. 3. α-methyl *p*-tyrosine treated. The number of corpora lutea is similar to that shown in the control ovary. Many large follicles are seen. The interstitial tissue is well developed. × 15.

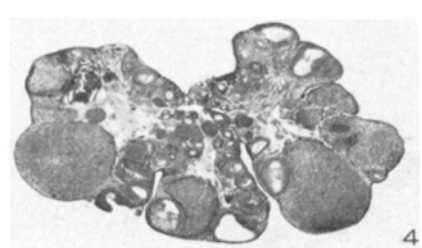

Fig. 4. α-methyl *p*-tyrosine + DOPA treated. 6 corpora lutea and many secondary follicles are observed. The interstitial tissue shows a normal appearance. × 15.

conclusion of this and other experiments reported recently[6] is that catecholamines stimulate the secretion of luteinizing hormone and inhibit luteotrophin.

Resumen. La administración continuada de α-metil *para*-tirosina, un inhibidor de la síntesis de catecolaminas, impide la hipertrofia compensadora del ovario en ratas. Este efecto es prevenido por la simultánea injección de DOPA, un precursor de las catecolaminas. El grado de la hipertrofia compensadora del ovario no fue modificado por la *p*-cloro-fenilalanina, un inhibidor de la síntesis de serotonina. El estudio histológico de los ovarios de los animales tratados con MPT sugiere que ésta bloquea la ovulación, no modificando aparentemente la secreción tónica de las gonadotrofinas FSH y LH.

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Accumulation of 5-Hydroxydopamine in 5-HT Storage Organelles of Cat Platelets and Concomitant Changes in Platelet Aggregation

Pretreatment of cats with 5-hydroxydopamine (5-HO-DA), an amine acting as a 'false' sympathetic transmitter[1], leads to the accumulation of a highly osmiophilic substance in the vesicles of adrenergic nerve terminals[2] representing 5-HO-DA and possibly its β-hydroxylated and/or *O*-methylated metabolites[1,2]. In blood platelets of different species 5-hydroxytryptamine (5-HT) is mainly localized in specific subcellular organelles and can be visualized by electron microscopy as a highly osmiophilic material[3–5].

In platelets of cats pretreated with 5-HO-DA, the 5-HT storage organelles appeared to contain much more osmiophilic material than under normal conditions. It seemed therefore to be of interest whether 5-HO-DA is accumulated in platelets and whether it displaces 5-HT from the storage organelles as it displaces norepinephrine from the vesicles of adrenergic nerve endings[1,2]. In further experiments we studied whether the accumulation of 5-HO-DA in platelets inhibits their aggregation as is the case after accumulation of 5-HT[6].

Cats of either sex were anaesthetized with 40 mg/kg of sodium pentobarbitone and 20–30 ml of blood were collected from the femoral artery into citrate as described previously[6]. After this first blood collection 20 mg/kg of H^3-5-HO-DA (labelled at the α- and β-C-atom of the side chain, specific activity 0.158 mC/mg) were injected i.v. and further blood samples were collected 30 and 60 min later. Each blood sample was centrifuged with 175 *g* for 10 min at 20–25 °C. The supernatant platelet-rich plasma was used to determine (1) platelet number and platelet volume with a Coulter Counter[7]; (2) the 5-HT content in isolated platelets[8]; (3) platelet aggregation produced by adenosine diphosphate (ADP) and 5-HT measured by a turbidimetric method[9,10]; (4) the radioactivity of 5-HO-DA and its metabolites in isolated platelets (centrifugation of platelet-rich plasma with 3200 *g* for 15 min) and platelet-poor supernatant plasma. After deproteinization with 0.4 *n* $HClO_4$ the radioactivity present in platelets and platelet-poor plasma was separated into acidic and alkaline (amine) fractions on Dowex 50 WX-4 columns. After acetylation the amines were further analyzed by paper chromatography as described previously[1]. In 2 experiments the platelets isolated before and after treatment with 5-HO-DA were prepared for electron microscopy as described previously[3].

Fine structural investigations of blood platelets of 5-HO-DA pretreated animals revealed notable changes in the aspect of the 5-HT organelles. The dense osmiophilic core of these organelles appeared to be on average much larger than those of platelets in control animals. In most instances the dense core now filled the organelle completely, whereas in controls only a partial filling is observed (compare Figure 1 with 2). In addition, after treatment with 5-HO-DA many 5-HT organelles possessed a worm-like, strongly osmiophilic appendix which sometimes surrounded an adjacent clear vacuole (Figures 2b to f). An analogous aspect occurred in control platelets also, although this was exceptional. The number of the 5-HT organelles, counted in ultrathin sections[3] was, if at all, only slightly increased: 116.5 ± 4.8% in platelets of animals 1 h after treatment with 5-HO-DA compared to 100 ± 5.5% in controls. With the exception of the 5-HT organelles no other fine structural changes could be detected in the platelets after 5-HO-DA administration.

Uptake into blood platelets of 5-hydroxydopamine (5-HO-DA) injected i.v. in relation to the 5-hydroxytryptamine (5-HT) content of the platelets

	5-HO-DA μg/10^8 platelets (mean ± S.E.)	5-HT μg/10^8 platelets (mean ± S.E.)
Before 5-HO-DA	–	1.47 ± 0.30
30 min after 5-HO-DA	2.36 ± 0.58	1.31 ± 0.25
60 min after 5-HO-DA	2.02 ± 0.55	1.25 ± 0.22

20 mg/kg 5-hydroxydopamine (1/10 as 3,4,5-Trihydroxyphenethylamine-α,β-$^3H_2$) was injected i.v. into 6 cats after a first blood collection. More blood was collected 30 and 60 min after this injection.

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