pyridine (2/1). Ingenol triacetate **7** was crystallised from methanol, m.p. 196 °C; m.s. (electron impact): m/z 474 ($M^{+\cdot}$, 2.5%, $C_{26}H_{34}O_8$), 456 (2%), 414 (16%), 354 (40%), 312 (93%), 294 (86%) and 121 (100%); IR (solid film KBr disc): γ_{max}, 3430, 1740, 1705 and 1640 cm^{-1}; C.D. (methanol): (210 $[\theta] = +5148$, 224 $[\theta] = -20064$, 298 nm $[\theta] = +3003$).

The acyl groups in the case of compounds **1, 2** and **5** were assigned to the C-3 position on the basis of the comparison of their NMR-spectra to those of other ingenane diterpenes[6].

The irritant dose 50% (ID_{50}) on mouse ears was determined for the 3 pure compounds (figure) both 2 h and 24 h after application to the test animals. The onset of erythema was rapid and the majority of the compounds were biologically active in sub-µg-doses. In addition the erythema persisted for 24 h and was still measurable after this time. This is in contrast to the daphnane esters isolated from some *Euphorbia* species[13] and it is possible that ingenane esters cause more extensive tissue damage. Compounds **1, 2,** and **5** were also found to be about as potent as podophyllin in inhibiting the uptake of ^{3}H-thymidine by TLX/5 mouse lymphoma cells. These compounds therefore represent the irritant and cytotoxic constituents of *E. paralias,* but they may be readily separated from the commercially required hydrocarbon fraction by solvent partition.

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Serum dopamine-beta-hydroxylase activity in a new strain of spontaneously hypertensive rats[1]

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Summary. The serum dopamine-β-hydroxylase (DBH) activity is higher in 5-week-old rats of the Lyon Hypertensive strain than in rats of the Lyon Normotensive strain. This difference disappears in older animals when the hypertension is developed, while the DBH activity decreases in the both strains.

The noradrenaline synthesizing enzyme, dopamine-β-hydroxylase (DBH), is contained in the vesicles of the noradrenergic nerves and released with noradrenaline by exocytosis[3,4]. Therefore, this enzyme is present in the blood and the determination of the circulating DBH activity has been used as an index of the sympathetic nervous system tone[4].

Thus, it was of interest to measure this parameter in order to evaluate the participation of the peripheral noradrenergic nerves in experimental hypertension. The aim of this work was to study serum DBH activity in a new strain of spontaneously hypertensive rats[5] at different ages which characterize the development of hypertension.

Male rats of the 13th generation of the Lyon Hypertensive strain (LH rats) or Lyon Normotensive strain (LN rats) were used at the ages of 5, 9 and 21 weeks. The day before sacrifice, the systolic blood pressure was measured by a tail cuff plethysmographic method (Narco Biosystem) in preheated (10 min at 36 °C) rats. The animals were sacrificed by a blow on the head; then blood was obtained by cardiac puncture, centrifuged (+4 °C) and the serum used for the determination of the DBH activity.

The DBH activity was measured according to a modification of the method of Molinoff et al.[6]. The assays were performed on 10 µl of 5-fold diluted serum; 5 µl of 25×10^{-5} M $CuSO_4$ were added, followed by 10 µl of a reaction mixture containing: 2 µl of 0.5 M sodium fumarate (pH = 5.0), 2 µl of 12 mM sodium ascorbate, 1 µl of 7.5 mM pargyline, 1 µl of catalase (260 units), 2 µl of 0.4 M sodium acetate buffer pH = 5.0 and 2 µl of 6.25 mM tyramine. Boiled serum was used for the blanks. The incubation was carried out at 37 °C for 45 min and was stopped by cooling the tubes in an ice-water bath. Then, 10 µl of a 2nd reaction mixture were added, containing 5.5 µl of 1 M Tris HCl buffer pH = 8.6, 2.5 µl of 0.1 M sodium ethylenediaminetetracetate, 1 µl of phenylethanolamine-N-methyltrans-

Systolic blood pressure, body weight and serum dopamine-β-hydroxylase activity in spontaneously hypertensive (LH) and normotensive (LN) rats at different ages

Age (weeks)	Rat strain	Systolic blood pressure (mm Hg)	Body weight (g)	DBH activity (nmole/h/ml)
5	LN	103.1 ± 2.3	87 ± 4	15.14 ± 1.07 (10)
	LH	114.8 ± 1.8**	91 ± 9	19.36 ± 1.49* (9)
9	LN	120.8 ± 3.1	216 ± 8	4.58 ± 0.44 (14)
	LH	145.6 ± 5.4***	233 ± 9	4.87 ± 0.47 (14)
21	LN	128.5 ± 4.9	320 ± 8	2.98 ± 0.26 (7)
	LH	173.5 ± 4.1***	390 ± 10***	3.29 ± 0.38 (7)

The values are expressed as mean ± SEM and the number of animals is in brackets. The DBH activity is expressed in nmoles of tyramine transformed per h and per ml of serum. The statistical differences between LN and LH rats at the same age were determined by student's t-test for unpaired data and are indicated: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

ferase partially purified according to Saelens et al.[7] and 1 µl (0.5 µCi) of S-adenosyl methionine methyl-^{3}H (sp. act. 10 Ci/mmole). The reaction was carried out at 20 °C for 10 min, and stopped by the addition of 65 µl of 0.7 M sodium borate buffer pH = 10.0. Then 900 µl of a mixture of toluene and isoamyl alcohol (3 v/2v) were added; after shaking and brief centrifugation, 600 µl of the organic phase was transferred to a scintillation vial and evaporated at 65 °C under a stream of air. The residue was redissolved in 1 ml of absolute ethanol; 10 ml of toluene containing 0.4% of diphenyloxazole and 0.01% of diphenyloxazolylbenzene were added and the radioactivity was measured by liquid scintillation.

As indicated in the table, the systolic blood pressure was higher in LH than in LN rats at the 3 different ages studied. The body weight was always higher in LH rats than in LN rats, the difference being significant only in 21-week-old animals.

In 5-week-old LH rats, the serum DBH activity was significantly higher (+28%, $p < 0.05$) than in age matched LN rats. This difference disappeared in 9- and 21-week-old rats, while in the both strains the serum DBH activity decreased with age (see table). In addition, a positive linear correlation ($r = 0.44$; $p < 0.05$, $n = 19$) was observed between the serum DBH activity and the systolic blood pressure of 5-week-old LH and LN rats.

Such an early increase in serum DBH activity has also been reported in young spontaneously hypertensive rats of the Japanese strain[8,9] and interpreted as reflecting an activation of the sympathetic nervous system. The same mechanism could be the origin of the increase in serum DBH activity observed in the 5-week-old hypertensive rats of our strain. Furthermore such an activation of the sympathetic nervous system is likely to be present in our rats since their enzymatic capacity to synthesize the peripheral catecholamines, as well as their urinary output of catecholamines, was increased[10-12].

In addition, it is interesting to note that serum DBH activity, as well as the other biochemical parameters related to the sympathetic nervous system[10-12] is only increased in 5-week-old LH rats, and returns to normal values in older animals, i.e. when the high blood pressure is fully developed. Such an evolution pattern, together with the existence in young LH rats of a positive correlation between the serum DBH activity and the systolic blood pressure values suggests that the sympathetic nervous system could be involved in the development of the genetically linked hypertension in that strain.

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Effects of bicuculline and chlordiazepoxide on locomotor activity and avoidance performance in rats

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Summary. Bicuculline, at a dose of 1 mg/kg which, per se, failed to change locomotor activity in rats, counteracts the facilitating effect induced by chlordiazepoxide (10 mg/kg). Conversely, bicuculline (1 mg/kg) does not modify the decrease of motor activity and the disruption of avoidance performance induced by this benzodiazepine derivative (20 mg/kg).

Several reports seem to support the possibility that GABAergic mechanisms are involved in some behavioral effects of benzodiazepines. Particularly, it has been shown that GABA receptor blocking agents, such as picrotoxin and bicuculline, antagonize the effects of benzodiazepine derivatives on conflict schedules in rats[1,2]. Furthermore, picrotoxin has been shown to counteract diazepam-induced amnesic-like activity in rats[3].

On the other hand, contradictory results have also been reported. Gardner et al.[4] found that the effects of GABA mimetic agents on locomotor activity in rodents differed considerably from those of benzodiazepine derivatives. Moreover, it has been shown that picrotoxin does not modify the depressant effect induced by diazepam on locomotor activity in rats and in mice[3,5]. Furthermore, Cook and Sepinwall[6] completely deny the possibility of an involvement of GABA in the anti-conflict activity of benzodiazepines. In the present experiment, bicuculline and chlordiazepoxide were employed in order to further evaluate the role of GABA in the effects of benzodiazepines on spontaneous locomotor activity and avoidance performance in rats.

Methods. Locomotor activity. The experiments were carried out on naive male Wistar rats weighing 240–260 g. The apparatus used in the present work was similar to that previously[7] and more recently[8] utilized for studying the spontaneous locomotor activity of mice. It consists of a series of toggle-floor boxes encased in a sound-attenuating chamber. Each box is divided into 2 compartments connected by an opening of 21 × 22 cm. For each rat, the number of crossings from 1 compartment to the other