heart and adrenal catecholamine concentration is as yet to be determined.

The heart is the most sensitive tissue to the depleting action of the (—)-isomer with greater than 90% reduction of norepinephrine at the 25 mg/kg dose. Brainstem and adrenal glands were lowered 30 and 50% respectively at the 100 mg/kg dose. This depleting action on the adrenals has been confirmed by DAIRMAN[13] and has not been observed with 6-hydroxydopamine[14]. In contrast, both isomers failed to lower brainstem serotonin concentration which is similar to the effects of 6-hydroxydopamine[2].

The results with the optical isomers of 6-hydroxydopa suggest some interesting possibilities in utilizing one of these compounds as experimental pharmacological tool. First, (—)-6-hydroxydopa at a dose of 25 mg/kg for 2 days can markedly deplete peripheral stores of norepinephrine without altering central levels. Alternatively, utilizing (—)-6-hydroxydopa with a peripheral decarboxylase inhibitor such as N^1-(DL-seryl)-N^2-(2, 3, 4-trihydroxybenzyl)hydrazine[12], peripheral depletion of catecholamines can be kept at minimal levels while central depletion is pronounced. Finally, (—)-6-hydroxydopa (100 mg/kg) administered peripherally can deplete brain norepinephrine without altering brain serotonin content.

Zusammenfassung. Die optischen Isomeren von 6-Hydroxydopa wurden synthetisiert und ihr Effekt auf den Katecholaminspiegel verschiedener Organe in der Ratte bestimmt. Dem (—)-Isomeren gegenüber waren Herz und Nebennieren äusserst empfindlich und im Gehirn wurde der Norepinephrinspiegel erniedrigt.

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Electrocardiographic and Behavioral Effects of L-DOPA in the Guinea-Pig

Since 1960[1] the guinea-pig has been shown to be susceptible to psychopharmacological procedures, particularly to the deconditioning effect of psychotropic drugs[2–12].

It is known that L-DOPA, beside representing a new and very effective agent for the treatment of parkinsonism, may influence human and animal behavior[13]. Perhaps the behavioral effects of L-DOPA may contribute in some degree to the efficacy of the drug in parkinsonism. Therefore, in the present note we report some results of research in progress on the behavioral effects of L-DOPA in the guinea-pig. A specific deconditioning effect[6,11,12] of the drug in this animal could support the hypothesis that L-DOPA has psychotropic properties, in addition to antiparkinson action.

On the other hand, many Parkinson patients treated with L-DOPA develop side effects, including psychoneurological and cardiological alterations[13]. Thus the purpose of our research was also to investigate and compare the effects of L-DOPA on spontaneous behavior and ECG in guinea-pigs[2,3]. Since the side-effects and toxicity of L-DOPA per os in man seem to be mainly connected with impurities contained in a 'preparation of doubtful quality'[13], in our experiments we have used L-DOPA supplied by Roche of Switzerland (LARODOPA), which 'until recently was the only L-DOPA approved by the Committee' on the Safety of Drugs[13].

For the clinical implications of our experimental results we have used the same route of administration and a dose pro kg close to the daily dose pro kg usually employed in Clinics for the L-DOPA treatment of parkinsonism.

Methods and results. Effects on spontaneous behavior and ECG. In 15 male guinea-pigs weighing 420–650 g, L-DOPA in single oral dose of 10-100-1000 mg/kg (5 animals for each dose) did not induce any changes in spontaneous behavior or ECG recorded before and 1–24 h after the administration.

In another experiment, 4 male guinea-pigs, each weighing about 500 g, were subjected first to administration of 1000 mg/kg per os of L-DOPA followed 24 h later by a second administration of 3000 mg/kg per os of L-DOPA. After the first administration no behavioral changes were observed, while increased diuresis, tremors and depression were found in all the 4 guinea-pigs after the second administration. 1 guinea-pig died 40 h after the second administration, while the other 3 animals survived, recovering from the behavioral changes in a few hours following the second L-DOPA administration.

No significant changes were found in the electrocardiographic records made in D_2 before, 1 h and 24 h after each administration of L-DOPA. No more deaths or behavioral changes occurred in the 18 guinea-pigs during the week following both experiments.

Effects on avoidance conditioning. 12 male guinea-pigs weighing about 500 g each were conditioned to an avoidance situation by the method described by MA-

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RINO[2,3]. 6 animals were given 1000 mg/kg L-DOPA per os/day. During the treatment, and for the 3 days following its interruption, the animals were regularly subjected to the conditioning sessions[2,3]. The L-DOPA was administered in each animal 120 min before the conditioning session.

Another group of 6 animals was kept as a control without any pharmacological treatment and was subjected to the conditioning sessions as previously described[2,3]. In the 12 animals the interval between sessions was 24 h. Results are reported in the Table. No change in the 'escape responses' (unconditioned responses) was found during the reduction of conditioned responses induced by the drug.

Deconditioning action of L-DOPA in the guinea-pig

Guinea-pig No.	Treatment	0	1st	2nd	3rd	4th	5th	6th day treatment
1	no	90	90	90	100	90	90	90
2	no	80	90	80	90	80	90	90
3	no	100	90	90	100	90	90	90
4	no	90	100	80	90	90	80	90
5	no	80	85	85	90	90	85	85
6	no	100	90	95	100	100	100	100
7	L-DOPA	100	15	25	30	80	80	90
8	L-DOPA	90	20	20	20	90	90	90
9	L-DOPA	95	0	0	0	90	95	90
10	L-DOPA	85	0	15	0	80	85	90
11	L-DOPA	90	10	10	10	90	95	90
12	L-DOPA	80	0	0	0	80	85	80

Percentage of conditioned responses (C.A.) to the avoidance situation in 6 control-guinea-pigs and in 6 guinea-pigs treated with L-DOPA (1000 mg/kg/os/die) for 3 successive days, after the maximum C.A. was reached and maintained for 3 successive sessions. 0, the last conditioning session before beginning the L-DOPA treatment. 1st-2nd-3rd, days in which the session was performed 120 min after the L-DOPA administration. 4th-5th-6th, days without L-DOPA administration.

The deconditioning effect of L-DOPA was readily reversible after the end of the treatment. The deconditioning dose we have used in the guinea-pig is only 15 times higher than the middle therapeutic daily dose employed in man (= about 67 mg/kg per os).

Therefore, a 3-day treatment with a daily dose of L-DOPA not affecting spontaneous behavior or unconditioned responses, is able to determine a deconditioning effect on avoidance in guinea-pigs.

Conclusions. The effects of L-DOPA administered per os on conditioning, spontaneous behavior and the electrocardiogram of the guinea-pig are reported and compared. L-DOPA is able to inhibit the conditioned behavior in doses per os without any effect on spontaneous behavior or motility. Moreover L-DOPA in doses up to 15 times higher than the average therapeutic daily dose pro kg used in man (TDD), does not alter the ECG of the guinea-pig. We did not observe ECG changes even after a total dose pro kg 60 times higher than TDD, which induced behavioral alterations in the 4 treated animals and one death.

The present results may indicate psychotropic properties for L-DOPA and do confirm the low toxicity and cardiotoxicity of the drug, when supplied in a pure preparation of quality[12].

Zusammenfassung. Verabreicht man L-Dopa (1000 mg/kg per os) an Meerschweinchen, so kann dadurch das konditionierte Verhalten gehemmt werden, während das Spontanverhalten und die Motilität unverändert bleiben. Es sind keine Modifikationen des Elektrokardiogramms festzustellen, selbst dann nicht, wenn die 60fache mittlere therapeutische Tagesdosis gegeben wird.

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Development of Acetylcholine Sensitivity in Cultured Skeletal Muscle

The morphological stages involved in the transition from a mononuclear myoblast through a multinuclear myotube to a striated muscle fibre in culture have been established[1-7] but the factors influencing the differentiation process and the physiological development of the fibre remain the subject of intensive investigation. In this preliminary study, an attempt was made to determine the earliest stage at which the developing fibre would respond to acetylcholine chloride (Ach).

Materials and methods. Cell suspensions containing 10^5 cells per ml were obtained from the leg musculature of 10 day embryo chicks by the method of KONIGSBERG et al.[8]. Aliquots of 3 ml were pipetted into 50 mm plastic petri dishes previously coated with collagen[9]. Application of drugs to the cells was performed by diffusion from a micropipette of tip diameter about 10 μ. The micropipette was filled with a solution of drug in Hanks solution and positioned to within 10 μ of the cell or myotube membrane. Diffusion could be aided or prevented by applying pressure or suction to the micropipette.

Results. Application of Ach solutions to myoblasts and to forming myotubes when at the 3–10 nuclei stage (Figure 1) failed to elicit contracture. After the formation of myotubes containing discernable cytoplasmic filaments (5 days of culture, Figure 2), application of $10^{-4}M$ Ach solution initiated fibrillation, and at higher concentrations caused a contracture which lasted for several seconds, followed by slow relaxation. This response was evoked in all myotubes which passed within 50 μ from the tip of

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