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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the micropipette, i.e. those fibres which came into contact with virtually undiluted Ach solution. Sensitivity was manifested at all points along the myotubes. The onset of sensitivity coincided with the appearance of occasional spontaneous twitches occurring randomly in groups of myotubes.

After 11 days, by which time development to the striated state was well advanced (Figure 3), spontaneous fibrillation was a common event, and the fibres could be stimulated to fibrillate by diffusion of $10^{-7}M$ Ach. Diffusion of Hanks solution alone as a control had no effect on the fibres.

When the medium in the petri dish was replaced with medium containing 1 µg/ml D-tubocurarine chloride (DTC), the cells were not affected by diffused Ach even at concentrations of $10^{-2}M$.

Diffusion of DTC or decamethonium iodide (C_{10}) solutions from the micropipette evoked a cellular response. DTC at 1 µg/ml had no effect, but at 0.1 µg/ml it caused a single twitch with rapid relaxation in fibres cultured for 11 days. In similar fibres, diffusion of 100 µg/ml C_{10} caused a contracture which was maintained for the duration of observation (about 20 min). Lower concentrations of C_{10} had no effect on the fibres.

Discussion. Previous work with developed skeletal muscle in culture has shown that it was capable of contracture in the presence of Ach[10–12], but the stage of development at which sensitivity appeared remained unknown. It is evident from our preliminary results that the developing myotube is capable of exhibiting a response to Ach long before it attains the striated state. The con-

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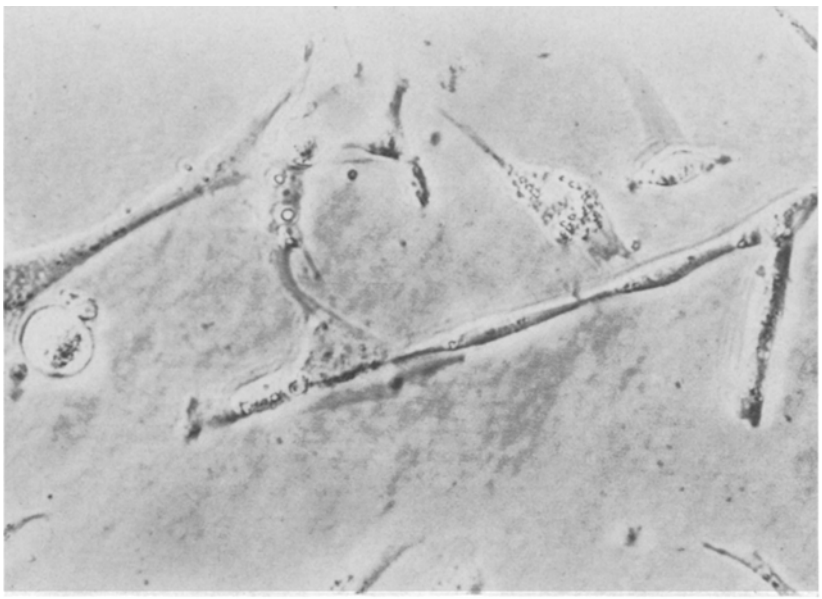

Fig. 1. Myoblasts aligning end to end and fusing to form a myotube. Contractions have never been observed in such cells at this stage. 3 day culture.

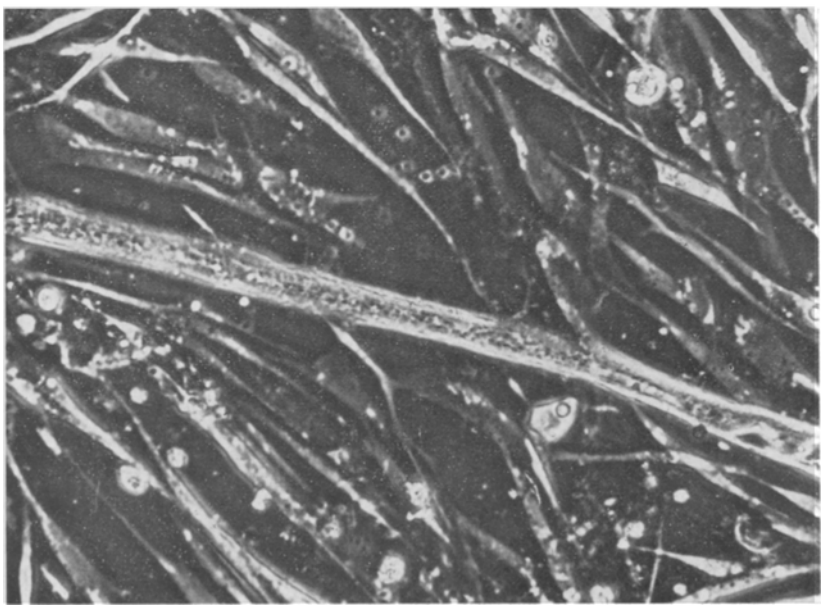

Fig. 2. Myotube with chains of central nuclei and longitudinal orientation of sarcoplasmic contents. This cell has begun to respond to $10^{-4}M$ Ach. 5 day culture.

Fig. 3. Muscle fibres showing cross striations. These cells are sensitive throughout their length to 10^{-7} *M* Ach. 11 day culture.

tractile mechanism is functional shortly after the rapid fusion of myoblasts into myotubes since spasmodic twitching of myotubes has been noted to commence between the third and the fifth day of culture[13–15].

Mononuclear myoblasts early in culture do not possess sarcoplasmic reticulum, T-tubules or myofilaments[16], and the process of cell fusion appears to precede the first appearance of organized filaments. Fluorescent antibody confirmation of the presence of myosin in myotubes was obtained after 4 or 5 days in culture[17] although random filaments have been observed in electron micrographs of younger cultures[16,18] along with the first elements of sarcoplasmic reticulum and T-system. Orientation of the myofibrils along the axis of the myotube immediately preceded the appearance of contractility. The myotube therefore contracts in response to administered Ach as soon as it has a functional though incompletely developed contractile mechanism.

Development of cross striations and a complete T-system is not completed until after 7 and 16 days in culture[18,19]. This subsequent development of the myotubes is accompanied by the observed increase in sensitivity over an 11 day period. Sensitivity to electrical stimulation has also been shown to increase with development in explants of muscle[20], and more recent work has shown that the potential across the myotube membrane increases as development progresses[21,22]. Whether the development of these properties is dependent on the same influence is not known.

Muscle cultured in this fashion can be considered analogous to preinnervated tissue in vivo. Foetal muscles are known to be sensitive along their length after innervation[23], and section of the motor nerve results in a spread of sensitivity from the end plate region to the entire fibre in adult muscle in vivo[24,25] and in vitro[26]. Sensitivity to Ach must therefore be a property of the entire muscle fibre which is modified by the presence of nerve.

The blocking agents employed exerted a predictable effect on the muscle fibres. DTC is generally regarded as a non-depolarising drug and at higher concentrations in the ambient medium, this property was demonstrated. At low concentrations it can cause depolarization and contraction in denervated muscle[27] and the possibility that this may be the case in cultured muscle has also been reported[11]. The results here noted confirm that this is so. C_{10} is a depolarizing drug, and the noted prolonged contracture of cultured fibres on application of C_{10} is in keeping with its known effects in avian muscle[28].

Résumé. Nous avons étudié in vitro la réaction des fibres musculaires, à l'action de l'acétylcholine, de la tubocurarine et du décaméthonium. L'acétylcholine a provoqué une contraction un peu après la fusion des myoblastes, avant que les fibres ne deviennent striées. La sensibilité des fibres à l'acétylcholine a augmenté avec le développement morphologique. Elle s'est manifestée le long des fibres et les drogues de blocage neuromusculaire ont agi comme prévu.

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