The experiments were carried out on adult male wistar rats. Brain slices were prepared according to the method already described[15] and were allowed 10 min in saline[16] at 37 °C. Propranolol (10 mg/kg) or dibenzyline (10 mg/kg) were injected intraperitoneally 30 min before the animals were sacrificed. The brain slices were incubated in the presence of noradrenaline (10^{-4} μM/ml), dopamine (10^{-4} μM/ml), histamine (10^{-4} μM/ml), serotonin (10^{-4} μM/ml), CAMP (10^{-3} μM/ml) and db-CAMP (10^{-3} μM/ml) and after 10 min glycogen was extracted[16] and estimated[17] from the brain tissue.

The results obtained show that propranolol prevented the glycogenolytic effects of noradrenaline and dopamine in vitro, but not that of histamine and serotonin, neither that of CAMP and db-CAMP (Table I). On the other hand, dibenzyline did not block the glycogenolytic effects, either of noradrenaline or dopamine, or histamine and serotonin, as well as of CAMP and db-CAMP (Table II).

CHASIN et al.[18] were the first to show that, in 2 areas of guinea-pig brain, cerebellum and cerebrum plus brain stem, there is a type of receptor shown to be a classical β-adrenergic receptor for the control of CAMP levels. Our data indicate that, in cortex, caudate and thalamus of rat brain, there exists β-adrenergic regulatory unit of adenyl cyclase responsible for the level of CAMP and activity of glycogen phoyphorylase. On the other hand, there must be another type of regulatory unit for histamine, as was suggested[9,18] and perhaps for serotonin. An α-adrenergic regulatory unit most probably would not be involved in the process of glycogenolysis in the brain tissue of rat.

Résumé. On montre que le propranolol empêche les effets glycogénolytiques de la noradrénaline et de la dopamine, bien qu'il n'ait pas d'effet sur les actions glycogénolytiques de l'histamine, de la sérotonine, du 3′, 5′-AMP cyclique et de son dérivé dibutyrique. On en conclu qu'au niveau du cervau des rats l'effet glycogénolytique des catécholamines résulte de l'excitation des récepteurs adrénergiques et que les autres unités régulatrices les adénylcyclases sont responsables des effets de l'histamine et de la sérotonine.

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Effect of Adrenergic Amines on the Membrane Potential of Guinea-Pig Liver Parenchymal Cells in Short Term Tissue Culture

In recent years the effects of catecholamines on the membrane potential of liver cells of several species have been studied in situ[1] in perfused liver[2,3] and in tissue slices[4]. The present experiments show that it is possible to apply the iontophoretic method of drug application[5,6] to examine the effects of adrenergic agonists on isolated parenchymal cells maintained under tissue culture conditions. This approach has two main advantages, 1. it is possible to see individual cells and select the appropriate cell from which to record and 2. it allows a greater resolution of the time course of the responses.

Materials and methods. Guinea-pig liver parenchymal cells were isolated by the enzymic procedure of BERRY and FRIEND[7] using collagenase, 0.025%, and hyaluronidase, 0.05% (both Sigma Type 1). The isolated cells were incubated in plastic petri dishes containing Eagle's medium (Dulbecco's modification), 10% tryptose phosphate broth, 10% foetal calf serum, antibiotics and a fungicide (Nystatin).

For the electrophysiological measurements a Hepes buffered Eagle's medium was used in which the calcium concentration had been increased to 3 mM. Recording was done at room temperature (21–23 °C). Membrane potentials were measured using glass micro-electrodes filled with 2 M potassium citrate (resistance 35–100 $M\Omega$). Micro-pipettes for iontophoresis were filled with 0.5 M solutions of (−)-noradrenaline, (±)-amidephrine or (−)-isoprenaline.

Results and discussion. The cultures contained single cells and small groups of cells. Many were binucleate (Figure 1). Stable membrane potentials ranging from −25 to −40 mV could be recorded from both mononucleate and binucleate cells whether single or in clumps. These values are similar to those reported[4] for cells in slices of guinea-pig liver. Noradrenaline invariably hyperpolarized the cells[4], as shown by A in Figure 2. A puzzling feature was the rather long latency (1–8 sec) of the response, which often could not be appreciably reduced by altering the position of the drug pipette. This may mean that it is necessary to 'flood' the cell with noradrenaline to produce a response, perhaps because the receptor density is relatively low. This interpretation was supported by the finding that the sensitivity of the tissue (expressed in terms of the potential change (mV) produced per nano-coulomb releasing the drug) was low (rarely more than 0.3 mV/nC).

Records B and C in Figure 2 show responses to iontophoretic application of (±)-amidephrine, a sympathomimetic amine which has a selective action on the α adrenoceptors[8,9]. Hyperpolarizations were again observed, although larger pulses were required, and the responses seemed to be more 'spiky' (for an extreme example, see C) than observed with noradrenaline. On the other hand, the strong β-agonist (−)-isoprenaline caused hyperpolarizations only if even larger pulses ($1–6 \times 10^{-7}$ A for 200–500 msec) were applied. The potency difference between noradrenaline and isoprenaline, together with

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the effectiveness of amidephrine, makes it almost certain that α-receptors are concerned although the possibility that β-receptors could contribute to the hyperpolarization, or even to underlie it under certain circumstances, has not been excluded.

Twenty-four hours after isolation the sensitivity of the cells to adrenergic amines had fallen 5–10-fold to a level which was retained for at least the following 2 days, although the maximum response attainable seemed to have declined. It was found that the large pulses of drugs needed to produce any effects now sometimes caused the membrane potential to become unstable, and on occasion to oscillate. An extreme example is shown in Figure 2D which was recorded from a 2-day-cell 2–3 min after a series of pulses of (−)-isoprenaline (up to 10^{-7} A for 500 msec) each of which had produced only slight increases in membrane potential. The mechanism involved both in this response and in the fall in sensitivity with time remain to be studied.

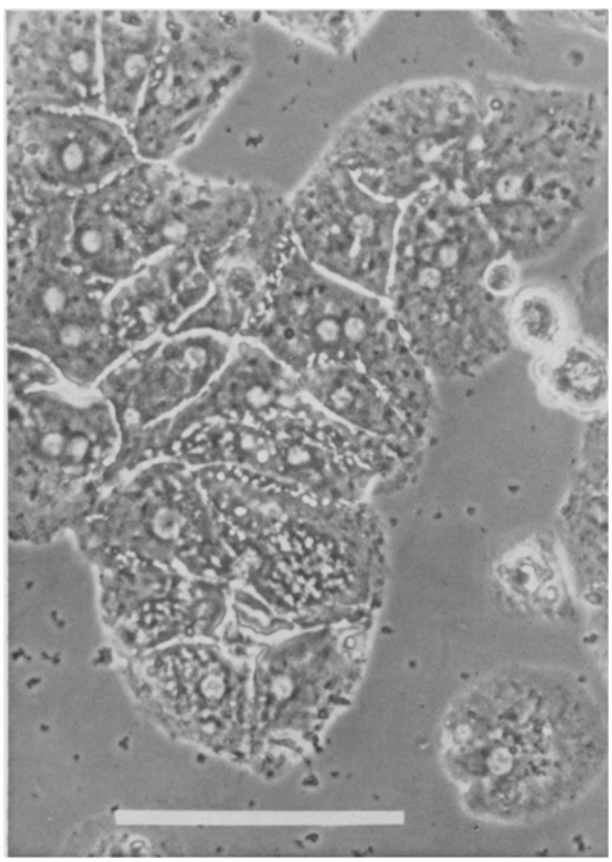

Fig. 1. Phase contrast photomicrograph of culture of parenchymal cells after approximately 22 h incubation (100 μm inset).

In summary, the present results show that it is possible to examine the electrical responses of isolated guinea-pig liver cells, maintained in short term tissue culture, to iontophoretically applied catecholamines. It is hoped that this approach may prove of value in the further study of the mechanism of action of catecholamines at the cellular level[10,11].

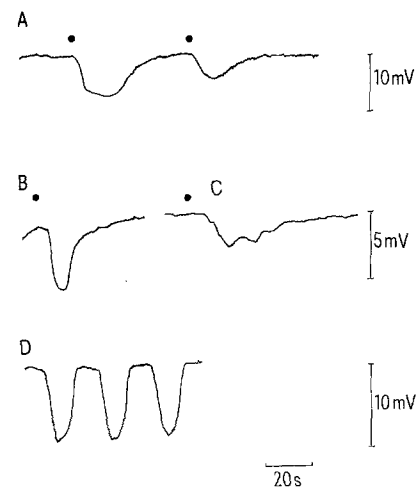

Fig. 2. Effect of iontophoretically applied adrenergic agonists on the membrane potential of parenchymal cells in tissue culture: A) Effect of (−)-noradrenaline (3.5×10^{-7}A for 50, then 25 msec) on a bi nucleate cell in a 6–8-h-old culture. B) and C) Effect of (±)-amidephrine (B) 3×10^{-7}A, 50 msec; C) — 9×10^{-7}A, 50 msec) in a 6–8-h culture. D) Oscillatory potential recorded 2–3 min after large pulses of (−)-isoprenaline had been applied to a cell in a 2-day-old culture. Room temperature throughout.

Zusammenfassung. Mit Hilfe der intrazellulären Mikroelektrodentechnik wird gezeigt, dass Parenchymzellen der Meerschweinchenleber in der Gewebekultur hyperpolarisieren, wenn sympathomimetische Drogen ionophoretisch appliziert werden.

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Zytophotometrische Untersuchungen an normalen Lymphknotenzellen des Schafes[1]

Die Zytophotometrie hat in den vergangenen Jahren in der Tumorpathologie vermehrt Anwendung gefunden. Durch Vergleich der Kern-DNS-Werte der Tumorzellen mit denen einer normalen Zellpopulation wird versucht, eine Aussage über die Charakteristik der Geschwulst zu treffen (SCHIEMER[2], SANDRITTER et al.[3,4]; SEIDEL et al.[5]; SANDRITTER[6–8]).

Im Zusammenhang mit unseren Untersuchungen über eine bei Schafen enzootisch auftretende lymphatische Leukose[9,10] interessierte vor allem auch die praktisch wichtige Frage, ob auf zytophotometrischem Wege die «Leukosezellen» von normalen lymphatischen Zellen unterschieden werden können. Als erster Schritt zur Beantwortung dieser Frage werden die oben genannten Metho-