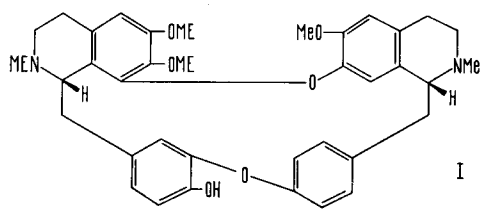
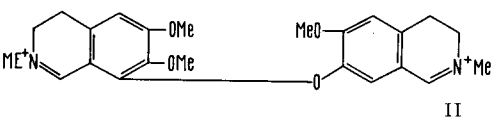
Treatment of pendulin in methanol with ethereal solutions of diazomethane and diazoethane yielded *O*-methylpendulin ($C_{38}H_{42}N_2O_6$) (M^+, 622), mp 150–152°, $[\alpha]_D$ + 210° and *O*-ethylpendulin ($C_{39}H_{44}N_2O_6$) (M^+, 636), mp 144–146°, $[\alpha]_D$ + 218°, respectively. The *O*-methyl derivative formed a hydrochloride mp 272–275° and a picrate mp 251–253°, and the *O*-ethyl derivative, a hydrochloride mp 262–264° (dec.) and a picrate mp 215–218°.

The molecular ion peak (M^+) at m/e 608 in the mass spectrum of pendulin is prominent. Other significant fragments appeared at m/e 607, 416, 396, 395, 381, 364, 349, 198, 175.5, 175, 174. This cracking pattern is characteristic of the oxyacanthine-berbamine type[7] of *bis*(benzyl)isoquinoline alkaloids. The characteristic double charged ion (II) m/e 198, which is the base peak in the spectrum and is accompanied by an isotopic peak at m/e 198.5, eliminates a methoxyl and a methyl radical to give an ion at m/e 175. The key ion at m/e 396, which looses an hydrogen atom to give an ion at m/e 395, is an ion always found in the mass spectra of the oxyacanthine-berbamine type of alkaloids[7], and its presence in the spectrum of pendulin is evidence that pendulin is isomeric with berbamine and oxyacanthine bases. From the fragmentation pattern, it also follows that all 3 methoxy groups present in the pendulin molecule are located in the tetrahydroisoquinoline moieties and the hydroxyl function in either of the 2 benzylic halves.

The NMR-spectrum of pendulin confirmed the presence of 40 protons in this molecule and the pattern of the spectrum was that of *bis*(benzyl)isoquinoline alkaloids[8]. The 2 N-Me functions gave rise to singlets at τ 7.38 and 7.68. Signals for 2 shielded *O*-Me groups appeared together as a singlet at τ 6.79 and the third *O*-Me group resonance was in the normal position at τ 6.25. 10 protons were responsible for the signals in the aromatic region of the spectrum. A shielded proton signal appeared as a singlet at τ 3.95 and a 2-proton singlet was present at τ 3.21. The remaining 7 protons (coupled *ortho*, *para* and *meta*) gave rise to multiplets between τ 2.60–3.80. 4 benzylic, 8-ring methylene and 2-ring methine proton signals were at τ 6.10, 7.12 and 6.58, respectively.



I



II

The NMR-spectra of *O*-methylpendulin and *O*-ethylpendulin were better resolved. The spectrum of *O*-methylpendulin had 4 *O*-Me signals at τ6. 02, 6.20, 6.62 and 6.72. A shielded aromatic proton was coupled with a *para* proton and appeared as a doublet at τ 3.94 (J, 1.5 cps). The 2 aromatic proton singlet at τ 3.08 was somewhat resolved. The other features of this spectrum were similar to those of the spectrum of pendulin.

In the NMR-spectrum of *O*-ethylpendulin, 3 *O*-Me signals were at τ 6.20, 6.63 and 6.78 and the *O*-Et triplet and quartet at τ 8.52 (J, 7 cps) and τ 5.82 (J, 7 cps), respectively. Other features of this spectrum were the same as in the spectrum of *O*-methylpendulin.

Bick et al.[8] have correlated chemical shifts of methoxyl functions with the stereochemistry of *bis*coclaurine bases. When the 2 coclaurine moieties of these bases are paired (+ −) or (− +), the 6′-methoxyl resonance has a chemical shift of τ 6.4, whereas with (+ +) or (− −) paired structures, the chemical shift is near τ 6.65. In *O*-methylpendulin and *O*-ethylpendulin the 6′-methoxyl function resonates at τ 6.62 and 6.63 respectively and therefore, suggests that pendulin exists in *Cocculus pendulus* in either (+ +) or (− −) forms.

The fact that pendulin exists in the (+ +) forms was confirmed by sodium and liquid ammonia reduction of *O*-ethylpendulin. A non-phenolic and a phenolic compound were isolated and found identical with (+) *O*-ethylarmepavin[9] and (+) N-methylcoclaurine[10], respectively. A *bis*coclaurine base pycnamine has recently been isolated from the roots of *Pycnarrhena manillensis*[11] and shown to exist in the (− −) form. Pendulin and pycnamine are, therefore, most probably optical isomers.

Zusammenfassung. Ein Biscoclaurin Alkaloid, Pendulin ($C_{37}H_{40}N_2O_6$), mp 192–194°, $[\alpha]_D$ + 265°, wurde aus Blättern und Stamm von *Cocculus pendulus* Diels isoliert und seine Struktur (I) inklusive der sterischen Anordnung an beiden Asymetriezentren aufgeklärt.

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Central Drug Research Institute, P.B. 173, Lucknow (India), 30 July 1969.

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Slow Spontaneous Signals from Brain Tissue Culture

Spontaneous signals have been reported from in vitro brain preparations of most classes of animals[1]. This paper describes spontaneous slow signals from 14-day-old chick embryo telencephalic explants. These may arise in dendrites or in glial cells secondary to neuronal activity.

Material and method. The chamber described previously[2] had a second 90 μ platinum (gross reference) electrode. A thin 1–2 mm diameter slice of right posterior pole of 14-day-old chick embryo telencephalon was placed on the bare tip of the gross recording electrode in the angle be-

tween sintered glass and roof of chamber. It was kept humid at 35 °C and used during its second culture day.

Glass microelectrodes containing 3*M* KCl (tip diameters 1 μ, impedance 10–50 megohms) detected signals which were amplified by Medistor A-35B negative capacity electrometers and Dana 3400 DC amplifiers. Grass P511R AC amplifiers were used for gross electrode signals. Signals were recorded on Hewlett-Packard Sanborn 3907B Magnetic Tape System at 15 in/sec and visualized on Tektronix 502A oscilloscopes or T.I. oscilloriters.

Histology. Active explants teased in nutrient fluid[1] were compressed between a glass slide and coverglass by 500 g for some min. Sealing the coverglass with Diatex[1] made a preparation suitable for phase-contrast microscopy for 2–3 h. Dendritic and axonal endings were better seen in teased cultures placed for 15 min at room temperature in equal parts of 1% gold chloride and saturated solution of mercuric chloride diluted 40 times with nutrient fluid.

Results and discussion. The explant (superficial corticoid area) showed its usual multilamellar structure[3] with histologically 'healthy' neurons (whose axonal and dendritic synaptic interconnections (Figure 1) created a net) and glial cells but seldom ependyma. 0.5% methylene blue in nutrient fluid stains neuronal granules without suppressing electrical activity[1], making them visible in thick explants. With this technic, good slow spontaneous signals were detected when the microelectrode tip was not in neurons or the immediate extraneuronal space. Methylene blue was not used in the other studies below.

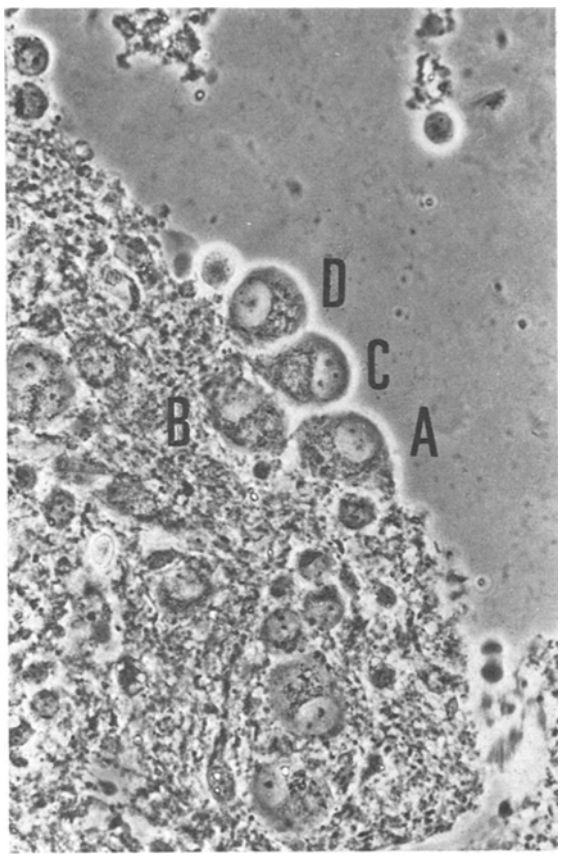

Fig. 1. Fresh teased preparation of a 1-day culture of 14-day-old chick telencephalon explant showing dendrite of (A) ending in a probable synaptic bouton on soma of (B) which sends a dendrite to the hillock and another to soma of (C). Possible dendritic connections of (C) to (D) are seen in the right and left corner. Phase contrast × 670.

A PDP4 computer was programmed to measure and produce frequency distribution histograms for duration of and intervals between thousands of AC amplified spontaneous signals. The duration histograms from each active explant had a mutual close similarity as did the histograms for intersignal intervals (Figure 2).

Microelectrode studies. The division of spontaneous signals[2] into short (1–3 msec, 10–50 mV), intermediate (5–500 msec, 5 μV–1mV) and long ($^1/_2$ to several min, 1–15 mV) duration types is inaccurate. Long duration signals are artefacts from overlapping and partial fusion of successive intermediate duration (slow) signals whose intersignal intervals are shorter than the signals. Spontaneous sequences of monophasic slow signals (Figure 3,A) equivalent to but simpler and larger than those detected by the gross electrode were detected. The gross electrode signals are smaller probably because of electrical shunting between electrode and frit. If only the tip of the recording gross electrode contacts the explant, signal magnitude approximates that from microelectrodes. The active foci in explants were small and sharply delineated; if 2 microelectrodes touch an explant less than 100 μ apart, only one may detect activity.

Successive microelectrode recordings at a linear series of sites 100–200 μ apart on the explant surface show changes in signal polarity. The number and form of signals in each sequence is constant for any one site but differs for each position of the tip (Figure 3). This 'reversal phenomenon' starts as a polarity change from 'positive-going' to 'negative-going' in the same first and last few signals in each sequence when the electrode is moved to a new site (Figure 3,C). At each subsequent position (Figure 3,D) more signals will have reversed in polarity until all are affected. The inverted signals are similar in number and form (although inverted) but smaller than equivalent non-inverted signals. The completeness of reversal and the distance needed to accomplish it vary from culture to culture and each active focus has one plane in which reversal is most clearly seen. If the microelectrode is returned across the surface of the explant in similar steps, the original polarities are regained. The stationary implanted gross electrodes detect no change in form or polarity of signal when the reversal is being detected by a microelectrode (Figure 3). Thus, signal polarity must be related to microelectrode position. Permanent gross electrodes may detect a partly inverted form of sequence which, in microelectrode studies, arises only from minute areas of an explant, so that the area of gross electrode pickup may be very small.

Simultaneous fast and slow signals are occasionally detected by gross electrodes but rarely by microelectrodes (due to the smaller field of pickup). Slow signals are then associated with a shower of fast signals or an increase in numbers of ongoing fast signals. Fast positive signals usually occur at the commencement of slow signals and fast negative ones at the end.

Slow signals may be continuously repetitive instead of in patterned sequences. Microelectrode exploration of a

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focus showing such activity, detected signals of about 10 msec duration at one site, 20 msec at a second and 60 msec at a third site. Both positive and negative going forms of these signals were also found. The 10 msec signals were simple peaks with slow decay and longer signals had subsidiary smaller peaks. Superposition of the simplest signals showed them to have the same duration but different levels of magnitude suggesting summing of simultaneous simple components. Superposition of complex signals showed secondary components at fixed intervals after the primary one, i.e. 10 msec peaks followed the 10 msec initial component at multiples of $2^1/_2$ msec until 30 msec and then more irregularly. Superposition of signals from gross electrode records showed similar relationships.

The resemblance between the duration histograms and between intersignal interval histograms shows that each explant produces the same class of signal, similarly spaced and presumably from similar sources. The slow signals are apparently combinations of simpler elements. The reversal phenomenon suggests stratification of the sites of origin of positive and negative signal forms, i.e. the positive may arise in neuronal somata and the negative in dendrites. However, away from the plane of the reversal phenomenon, the pattern of the spacing and magnitude of the signals within sequences is similar over areas of an active focus larger than a cell and its processes (except axons). Closely patterned sequences of such similarity are unlikely to be produced by contributions of signals from the various cells contacting the microelectrode tip at each site. Thus, the signals must come via the intercellular space or each random contact must bring the microelectrode tip into contact with a cell capable of generating a sequence containing positive and negative signals. The sharp delineation of foci supports the possibility of the cellular route. When the microelectrode tip has been seen during slow signals it has not been in, or in visible contact with, neurons. The only other cell type in stationary

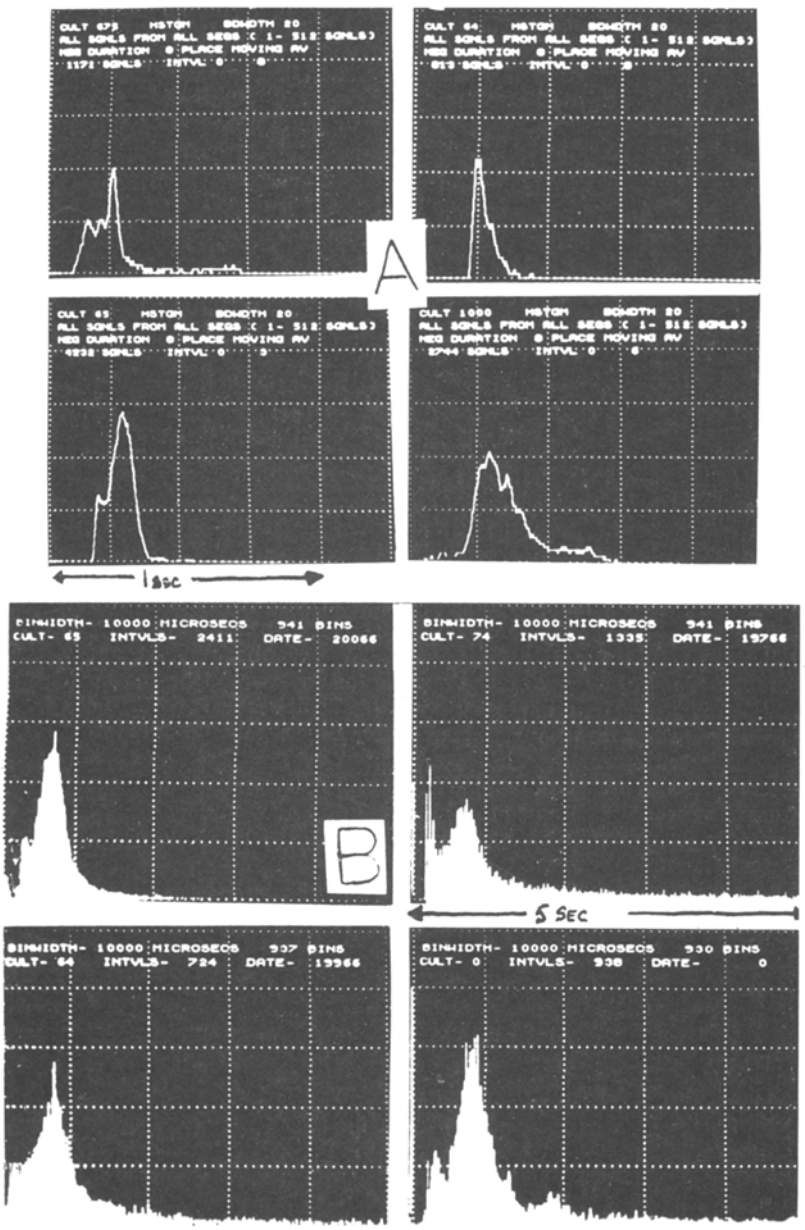

Fig. 2. (A) Frequency distribution histograms of durations of AC amplified slow signals from 4 different cultures of 14-day-old chick embryo telencephalon. (B) Frequency distribution histograms for intervals between AC amplified slow signals from 4 different cultures of 14-day-old chick embryo telencephalon. The similarities within each set of 4 histograms is obvious.

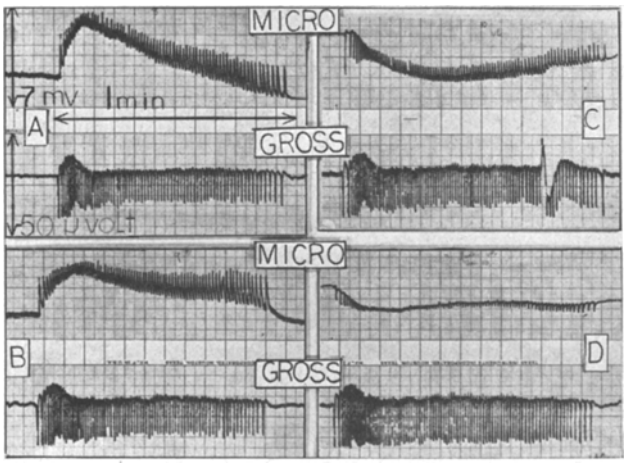

Fig. 3. Reversal of polarity of slow signals from microelectrode tip touching explant in 4 successive positions 100–200 μ apart in a straight line. The 1st, 3rd, 5th and 7th traces are DC records from microelectrodes at these positions and for each there is a simultaneous AC record from gross electrodes (2nd, 4th, 6th and 8th traces). The distortion at the end of record C is an artefact. The similarity between all gross records shows no change in the basic activity of the focus while a change in polarity of some of the signals in each sequence was recorded by the microelectrode.

contact with microelectrode in sufficient numbers is the glial cell. The slow signals have a definite resemblance to those produced by glial cells in response to associated neuronal action[4,5].

Resumén. La exploración con microelectrodos, de explantes telencefálicos de embrión de pollo, registra espontáneamente señales de dos tipos: lentas y rápidas, confirmando hallazgos similares previamente reportados con macroelectrodos. Observandose un patrón en la distribución de las señales lentas, las cuales son originadas por la misma fuente en cada explante pudiendo ser ésta, las células de glía o el producto de potensiales dendríticos.

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Fluctuations in Sarcomere Length in the Chick Anterior and Posterior Latissimus dorsi Muscles During Isometric Contraction

Recently, LARSON et al.[1] reported the detection and measurement of sarcomere fluctuation during isometric contraction of the frog sartorius using the optical diffraction patterns obtained by transmitting a laser beam through the muscle. In order to ascertain whether sarcomere fluctuation is a property peculiar to the frog sartorius it was decided that the laser beam diffraction method should be applied to some other muscles. In practice very few muscles are suitable for this kind of study as it is necessary to use a muscle that is very thin and which possesses sarcomeres of more or less uniform length. After a number of muscles had been tried it was found that the chick posterior latissimus dorsi muscle gave a good workable diffraction pattern. This muscle is a fast or phasic muscle whereas the adjacent anterior latissimus dorsi muscle is a slow or tonic muscle. It was possible to obtain a reasonable diffraction pattern from the anterior latissimus dorsi and, therefore, the extent of sarcomere fluctuation in the phasic muscle could be compared with that in the tonic muscle.

Materials and method. The posterior latissimus dorsi and anterior latissimus dorsi were dissected out from 20-day-old White Mountain Cross chicks. They were suspended in the muscle chamber as shown in Figure 1. The muscle chamber was filled with Krebs bicarbonate ringer solution at 38 °C, equilibrated with a 95% O_2/5%CO_2 mixture. The optical arrangement for recording sarcomere fluctuations during contraction was as follows: The laser beam (Optics Technology, Inc., Model 170, Helium-Neon gas Laser = 6328 Å, power 0.3 mw) was transmitted through the muscle in the muscle chamber and the diffracted beam was then focused into the lens of a 16 mm movie camera (Bolex 16 mm H reflex). Also the screen of an oscilloscope showing the stimulation pulses was reflected and focused into the top left-hand corner of the camera lens using several mirrors and lens. The length of the muscle was adjusted to its maximum resting length by lowering the bottom hook using the rack and pinion. It was then gently flattened between the glass plate and the muscle chamber wall using the other rack and pinion which moved the muscle chamber backwards and forwards in the horizontal plane. The laser beam was then switched on and moved up and down the muscle until a region was found which gave a good diffraction pattern. Care was taken not to expose any region of the muscle to the beam for more than a few seconds. The muscle chamber was then drained and the camera set in motion (60 frames per sec) and after a second or so the muscle was stimulated via the hooks from which the muscle was suspended, with a burst of 30 volt DC square wave pulses of 10 msec duration delivered at a frequency of 50 pulses per sec. The tension developed during the contraction was recorded using the myograph (Grass straingauge) with the output connected to a pen recorder (Dynograph Offner Type RS). After processing, the film was analyzed frame by frame on an isodensitometer (Joyce and Loebl Ltd., England) and the distance between the diffraction lines was measured and plotted for each individual frame of the film.

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