In the second part of the paper these principles are in practice extended to the use of multicomponent solvents, and the superiority of polyzonal over duozonal TLC is demonstrated. Polyzonal TLC is particularly useful for the direct chromatographic separation of mixtures comprising substances of highly different polarities. Chromatographic solvent demixing is counteracted by diffusion from and convection within the gas phase of the chromatographic chamber. Therefore, best results in polyzonal TLC are obtained when the horizontal covered plate technique[19] is used. For preparative separations based on the polyzonal principle, the use of the Fuchs column[23] is suggested.

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The Location of Microelectrode Tips in Nervous Tissues

In a previous paper we described the responses of cells in the cat cerebellar cortex to iontophoretically applied cholinergic drugs[1]. Because of the difficulties associated with identification of the area within which units were recorded, it was necessary to have a technique for placing small lesions in the cerebellar cortex that could be identified at the end of the experiment.

When using multibarrelled micropipettes, one barrel can conveniently be set aside for a lesion-making substance or else the substance can be incorporated in the recording barrel. Selected units can be defined by passing out the marker after recording from them, thus forming a small but histologically detectable lesion. Although a variety of techniques for locating the position of microelectrode tips has been described, none of these has been widely accepted[2]. We were unable to locate the position of electrode tips with any certainty after applying 90 V DC or 240 V AC to a barrel containing silver nitrate and treating the sections with ammonium sulphide[3]. Likewise attempts to pass silver nitrate by pressure were unsuccessful. A complex anion of copper and citrate is claimed to be superior to simple salts of heavy metals, such as lead and mercury, in causing lesions by the coagulation of protein, since use of the latter is frequently associated with the formation of a 'cap' at the electrode tip and consequently an inhibition of current flow[2]. We found, however, that in granule cell layer of cat cerebellum this solution produced lesions too large (300–800 μ) for our purposes, and in molecular layer or white matter it had little marking action.

A series of trials has led us to conclude that the iontophoretic application of hydrogen ion from a barrel containing hydrochloric acid invariably results in the formation, in granule cell or molecular layers, of discrete lesions, the diameter of which varies from 50 μ depending on the strength and duration of the current used for iontophoresis. Lesions in white matter were more diffuse. Acid-formed lesions do not 'cap' the electrode, and are little disturbed by further passage of the microelectrode, so that many marks can be left in one electrode track.

Figure A (i and ii) show columns of lesions in cat cerebral cortex and indicate the relationship of lesion size to

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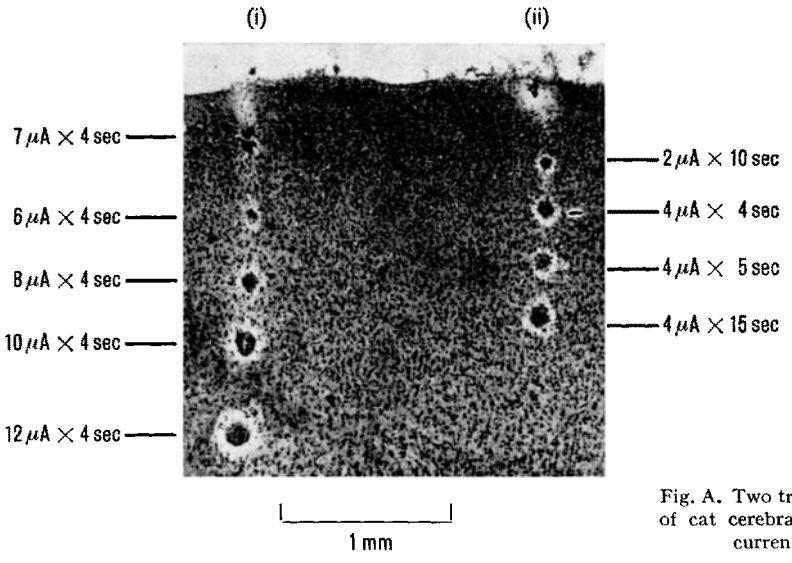

Fig. A. Two tracks, showing acid formed lesions, in the lateral gyrus of cat cerebral cortex. Strength and duration of electrophoretic currents indicated. Stained with Cresyl Fast Violet.

(current × time). Figure B comes from an experiment in cat cerebellar cortex and shows three lesions of 150–200 μ, centred on Purkinje cell layer, deep in granule cell layer and in molecular layer, respectively. These figures illustrate another advantage of acid lesions – the rapidity with which they can be produced. Exploration of the cerebellum is facilitated by traversing the long axis of folia at right angles, as shown in Figure B; this tends to make transitions between different regions more abrupt.

In order to pass currents of 1–10 μA from the hydrochloric acid-containing barrel of a pipette with an overall tip size of 10–12 μ, we find it necessary to use 0.1 N hydrochloric acid (barrels then have a resistance of 25–100 $M\Omega$). It is important to prevent the diffusion of hydrogen ions from the electrode during normal recording or passage through nervous tissue, since hydrogen ion has an excitant action on neurones[4]. A 'backing' current of 5 nA appears adequate for this purpose and the presence or absence of hydrochloric acid in the micropipette does not affect the excitability of cerebellar neurones. The efficacy of the lesioning can be judged by the degree of reduction in electrical activity at the recording site.

Fig. B. Three lesions in cat cerebellar cortex (tuber vermis); in Purkinje cell layer, granule cell layer and molecular layer (near a Purkinje cell layer). Strength and duration of electrophoretic currents: 7 μA × 15 sec, 4 μA × 10 sec and 16 μA × 10 sec. Stained with Luxol Fast Blue and Neutral Red.

Tracks marked with lesions can readily be located for subsequent sectioning if they are made in the same plane as two capillary-glass microelectrodes placed at either end of the row of tracks. It is also possible to pass hydrogen ion continuously whilst withdrawing the micropipette, thus leaving a continuous lesion that appears in the section as a line, the thickness of which varies with current and rate of withdrawal. Although valuable for some purposes, use of such a line for correlation of histological structure with electrical records would involve relying on depth readings from the micromanipulator. The results obtained during this series of experiments have shown that these are frequently unreliable.

Résumé. Des lésions pratiquées dans le tissu nerveux permettent de localiser histologiquement les régions explorées au moyen de microélectrodes. Des lésions de 50 μ ou plus peuvent être faites par des ions d'hydrogène introduits par iontophorèse avec une micropipette dont un des tubes contient de l'acide chlorhydrique. Les auteurs ont opéré sur l'écorce cérébrale et le cervelet du chat.

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Zur Methodik der elektrophoretischen Untersuchung der Proteine der Speicheldrüsensekrete

Die organischen Bestandteile des menschlichen Speichels können mit chemischen Methoden bestimmt werden[1–3], daneben kann ihre Trennung auch durch Elektrophorese erfolgen[4–12]. Zur Durchführung dieser Methode muss das Versuchsgut angereichert werden.

Bei 27 gesunden Versuchspersonen haben wir die 4 grossen Speicheldrüsen mit PVC-Röhrchen direkt sondiert. Nach Einführung der Sonden wurde zunächst 10 min gewartet, um den mechanischen Reiz der Sondierung abklingen zu lassen. Danach wurde der austretende Speichel als Ruhespeichel aufgefangen. Nach 45 bis höchstens 120 min waren 6 cm³ erreicht. Dann wurde die Speichelsekretion durch Lutschen frischer Zitronenscheiben stimuliert. Unter diesem alimentären Reiz betrug die Speichelsekretion etwa 1 cm³ pro Drüse und Minute. Der aufgefangene Speichel wurde als Zitronenspeichel bezeichnet. Anschliessend wurden zur Dämpfung des Parasympathikus 0,5 mg Atropin pro 70 kg Körpergewicht subkutan injiziert. 15 min nach der Injektion wurde der austretende Speichel als Atropinspeichel aufgefangen. Nach 35 bis 110 min waren etwa 6 cm³ erreicht. Danach wurde wiederum mit Zitrone stimuliert und der austretende Speichel als Atropin-Zitronenspeichel bezeichnet. Der unter diesen Bedingungen gewonnene Speichel ist bei allen Versuchspersonen relativ gleichmässig[13]. In manchen Fällen wurde anschliessend noch 1 Ampulle Prostigmin (R) i.m. injiziert.

Die unmittelbare elektrophoretische Untersuchung der einzelnen Speichelproben erbrachte keine einwandfreien Ergebnisse. Vor allem liessen die Zitronen-Speichelproben

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