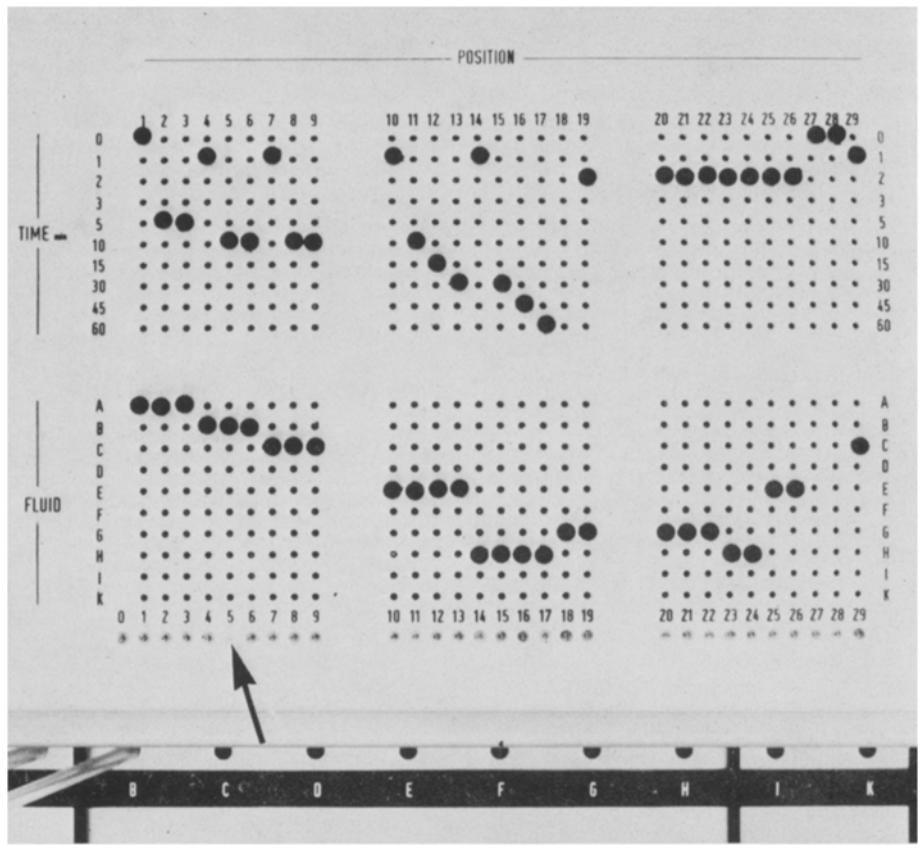

Fig. 3. Programme selecting panel. According to the programme selected in this illustration, the apparatus will perform the following steps: 1. step will be omitted due to time setting 0; 2 fluid A for 5 min; 3. fluid A (fresh) for 5 min; 4 fluid B for 1 minute (e.g. short rinsing); 5. fluid B for 5 min, etc. The light (→) indicates the actual step in progress (in this instance step No. 5). At step No. 18 the apparatus will remain operating (time positions without pin) until the switch 'Step' (not visible) is pushed in order to switch over to the following steps for self-cleaning.

fluid. Except for the mixing procedure, our apparatus operates on a similar principle. However, it has some different features: specimen baskets and tubes are disposable, containers of any size can be used as reservoirs, there is no need of pumps, and the reproducibility of the programmes is achieved by fixed time settings and premixed fluids.

Zusammenfassung. Eine neue, einfache Maschine zur automatischen Entwässerung und Einbettung elektronenmikroskopischer Präparate wird beschrieben. Bis zu 24 Präparate werden gleichzeitig in einer Wanne bewegt, deren Inhalt bis 29mal aus 10 verschiedenen Behältern entsprechend einem vorwählbaren Programm ausgewechselt wird. Die Maschine reproduziert zuverlässig und genau zahlreiche bisher von Hand ausgeführte Verfahren. Sie ist klein, einfach zu bedienen, kann über Nacht arbeiten und spart so viel Zeit.

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PRO EXPERIMENTIS

Optical Method for Measuring Water Flow with Automatic Recording

Flow of water across biological and synthetic membranes is a major feature of their permeability characteristics. Neurohypophyseal hormones can drastically change the water permeability of structures like distal nephron and amphibian epithelia. These latter membranes have been widely used both as a model of the mammalian kidney and as a convenient in vitro system to study water transport across polar epithelia.

Measurements of water flow across toad bladder and frog skin have always presented technical difficulties. Three main types of technique have been proposed: 1. gravimetric methods[1–3], 2. a constant volume technique with automatic recording[4], and 3. a volume flow technique employing an horizontal pipette[5]. The movement of a meniscus in an horizontal pipette or calibrated capillary is an almost ideal technique to measure water

flow by virtue of simplicity and paucity of artifacts. Its major disadvantage has been the difficulty in obtaining an automatic, continuous recording of the displacement of the meniscus. An optical method has therefore been developed that obviates such a disadvantage.

An horizontal pipette is attached to a chamber in which the membrane is mounted. The apparatus for continuous monitoring of the movement of the meniscus is schematized in Figure 1A. The main parts of the system are: a) A motorized carriage driving a light source, a photosensitive element (eg., a photocell) and a pen. b) A relay between the optical system and the carriage motor. c) A recording device, eg., a strip chart recorder. The moving carriage consists of a metal piece with different optical and recording elements attached. This assembly slides on a rail and is driven by an ordinary electrical motor and a precision-cut, steel lead screw. The motor speed is adjustable and greater than the maximal estimated speed of the meniscus movement. The following is an example of a suitable carriage assembly: a) a 3.5 volts electrical bulb, with focusing lens, b) a photoresistance Philips ORP 60 and c) an ink pen.

The relay between the optical system and the carriage motor is schematized on Figure 2. The amplifying circuit is represented on the left part of the diagram. The signal coming from the photo-resistance ORP 60 is amplified in a cold-cathode tube, Philips Z 805 U, and then injected into the delay circuit by means of an electro-magnetic switch. The delay circuit, represented on the right part of the diagram, directly commands the electrical motor by means of a second electro-magnetic switch. Finally, the electrical motor sets into motion the carriage assembly on the lead screw. The delay time is adjustable up to 3 sec, according to the flow rate of the liquid inside the pipette, in order to smooth the recording.

The method takes advantage of the difference in optical characteristics between the meniscus and the zone of the pipette filled with liquid, as shown in Figure 1B by a photoresistance measurement. When the carriage is placed in front of the empty zone of the pipette, the photosensitive element will drive the carriage motor by means of the relay system to a position immediately adjacent to the meniscus. At this point, a sufficient amount of light reaches the photosensitive element, placed on an axis perpendicular to the pipette, and the motor is stopped. Movement of the meniscus towards the chamber will intercept the light source and will trigger the carriage motor until illumination of the photosensitive element again occurs. The movement of the carriage and that of the meniscus inside the pipette are parallel and of the same magnitude, as indicated by the arrows in Figure 1A. The movement of the carriage can be directly recorded on the chart of an ordinary pen recorder. The sensitivity of

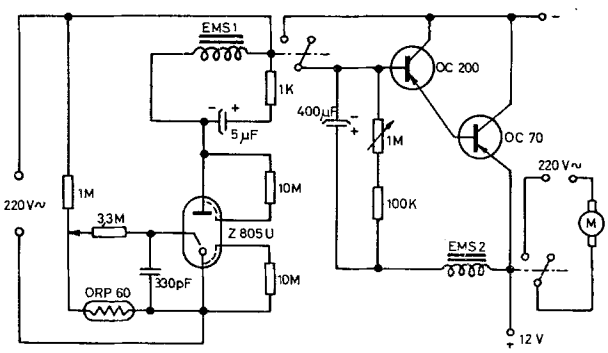

Fig. 2. Schematic drawing of the relay between the optical system and the carriage motor.
EMS_1, EMS_2, electromagnetic switches; M, carriage motor.

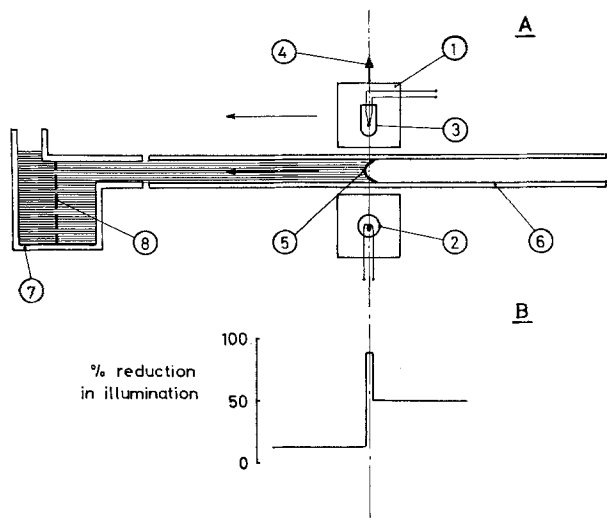

Fig. 1. A) Schematic representation of a system for measuring water flow with automatic recording: motorized carriage (1), which carries a light source (2), a photosensitive element (3) and a pen (4). The meniscus (5) moves inside the pipette (6) towards a chamber (7) divided by a membrane (8). When the meniscus intercepts the optical axis of the system, the carriage moves until illumination of the photosensitive element occurs. B) Differences in illumination of the photosensitive element produced by different zones of the pipette.

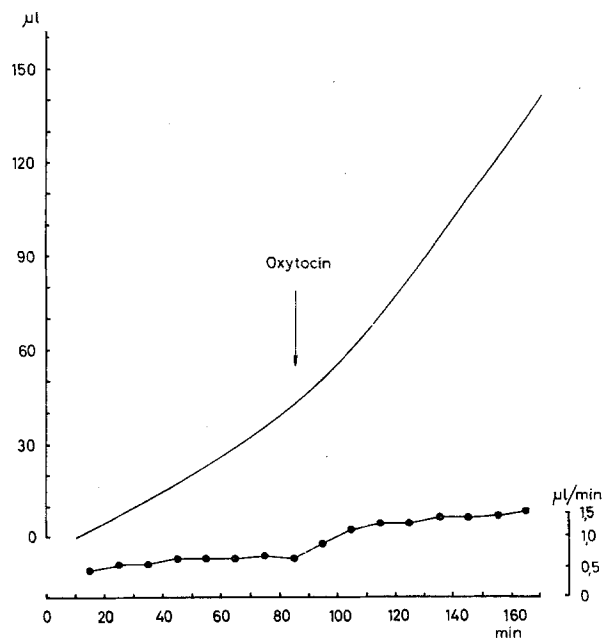

Fig. 3. Automatic, direct recording of water flow across frog skin, obtained with the system described in Figure 1. The derived curve at the bottom can also be obtained automatically.

[1] P. J. BENTLEY, J. Endocr. *17*, 201 (1958).
[2] R. M. HAYS and A. LEAF, J. gen. Physiol. *45*, 905 (1962).
[3] P. EGGENA, I. L. SCHWARTZ and R. WALTER, J. gen. Physiol. *52*, 465 (1968).
[4] J. BOURGUET and S. JARD, Biochim. biophys. Acta *88*, 442 (1964).
[5] I. S. EDELMAN, M. J. PETERSEN and P. F. GULYASSY, J. clin. Invest. *43*, 2185 (1964).

the method depends essentially on the characteristics of the pipette, fractions of a microliter being easily measured.

Figure 3 gives an example of the recording obtained in an experiment concerning the movement of water through the frog skin. The membrane was submitted to a concentration gradient of 1:10, established with a Ringer solution and the same solution diluted 10 times. During the control period a small flow of water passed through the membrane. Addition of oxytocin to the internal side of the skin promoted an increase in water flow as shown by the change in slope of the recording.

The recording shown in Figure 3 represents one of a number of potential methods of processing the information obtained. Addition of appropriate standard electronic circuits to the main system would allow, for example, the digital transformation of the data or its conversion into desired flow units.

The sharp optical transition between the meniscus and the zone of the pipette filled with liquid has thus been utilized in the design of a reliable and sensitive system permitting continuous, automatic recording of the movement of the meniscus. Information about the flow of water is hence provided without interference with the flow itself, by an optical device totally independent of the chamber + pipette system. Indeed, the apparatus described may readily be applied for liquid flow measurements in a wide variety of other systems[6].

Résumé. Une méthode optique d'enregistrement automatique de flux d'eau à travers des membranes biologiques et synthétiques est décrite. Le mouvement d'un ménisque à l'intérieur d'une pipette est suivi de manière continue à l'aide d'un dispositif photosensible, sans interférence avec le flux lui-même.

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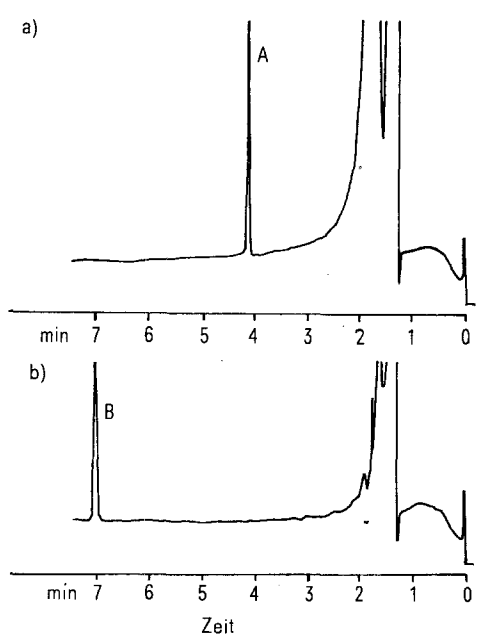
Ultramikromethoden. Hydrolyse von Essigsäureestern

In der 2. Mitteilung dieser Reihe[1] wurde eine Ultramikromethode zur Acetylierung von Alkoholen beschrieben. Acetylierungsexperimente waren mit je 1 Nanogramm der folgenden Alkohole durchgeführt worden: 1-Hexanol, 1-Octanol, 1-Decanol, 1-Dodecanol, 1-Tetradecanol, 1-Hexadecanol, 2-Octanol.

Das gleiche experimentelle Verfahren lässt sich auf die Hydrolyse der von den genannten Alkoholen abgeleiteten Essigsäureester anwenden. Als Hydrolysereagens dient 0,2 *N* KOH in 90%igem Methanol für die Acetate der primären Alkohole und 0,5 *N* KOH in 90%igem Methanol für 2-Octylacetat. In die Mitte einer Probenkapillare (Länge 40 mm, ä. ⌀ 0,92 mm, i. ⌀ 0,32 mm) wird mit Hilfe einer Staukapillare (Länge 60 mm, ä. ⌀ 0,9 mm, i. ⌀ 0,05 mm) auf die früher beschriebene Weise 1 ng des jeweiligen Esters und anschliessend eine etwa 0,5 mm hohe Säule des Hydrolysereagens aufgenommen. Die Reaktion ist in allen Fällen innert 30 min beendet. Da die Flüssigkeit in der Probenkapillare nach Ablauf dieser Zeit meist in Form einer dünnen Lamelle vorliegt, schliesst man eine Staukapillare an und erteilt der Lamelle einen oder mehrere Druckstösse, bis sie zusammenbricht.



a) Gaschromatogramm des aus 1 ng 1-Decylacetat entstandenen 1-Decanols (Peak A).
b) Gaschromatogramm von 1 ng 1-Decylacetat (Peak B).

Die aus der Hydrolyse hervorgegangenen Alkohole lassen sich hierauf gaschromatographisch nachweisen mit Hilfe der Methode und unter den Bedingungen, die früher bei der Untersuchung der entsprechenden Acetate angewendet worden sind. Die Figur zeigt als Beispiel ein Gaschromatogramm a) mit dem Peak A und ein Gaschromatogramm b) mit dem Peak B. Peak A stellt das aus der Hydrolyse von 1 ng 1-Decylacetat entstandene 1-Decanol dar, Peak B 1 ng des Ausgangsmaterials 1-Decylacetat.

In bezug auf die Gaschromatogramme der Hydrolyseprodukte von 1-Tetradecylacetat sowie 1-Hexadecylacetat ist zu erwähnen, dass einige Fremdpeaks auftreten, die dem Hydrolysereagens zuzuschreiben sind und sich nicht eliminieren lassen. Der Nachweis des 1-Tetradecanols bzw. 1-Hexadecanols wird dadurch etwas beeinträchtigt.

Benützte Probenkapillaren enthalten einen KOH-Rückstand und werden am besten so gereinigt, dass man das eine Ende in ein Schälchen mit Wasser eintaucht und über das andere Ende hinweg einen kräftigen Gasstrom, senkrecht zur Kapillarenachse, leitet. Die Kapillare füllt sich so mit Wasser, das man anschliessend ausbläst. Die Operation wird etwa zweimal wiederholt[2].

Summary. A hydrolysis method for nanogram amounts of acetic acid esters is described.

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[1] 2. Mitteilung: S. Huwyler, Experientia *28*, 718 (1972).

[2] Herrn Prof. Dr. M. Visconini danke ich für wertvolle Dirkussionen und dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung für die finanzielle Unterstützung.