PRO LABORATORIO

## Ein Mikro-Homogenisator

Neuere quantitative Methoden der Cytochemie, Histochemie oder Biochemie erfordern mitunter eine möglichst verlustfreie Zerkleinerung einzelner Zellen oder kleiner Gewebemengen. Da Homogenisatoren herkömmlicher Bauart diese Forderung im allgemeinen nicht hinreichend erfüllen, wird im folgenden ein Gerät beschrieben, welches eine verlustfreie Homogenisierung auch weniger Zellen erlaubt. Der Mikro-Homogenisator besteht aus einer einmal verdrillten Drahtschlaufe mit einer möglichst feinen Spitze, die in eine, das zu homogenisierende Material enthaltende Kapillare eingeführt und dort mit einem regulierbaren Motor zur Rotation gebracht wird. Die Verbindung zwischen Motor (Zahnbohrer) und Schlaufe wird durch zwei ineinander passende Polyäthylenschläuche hergestellt; der eine ist als Halterung fest auf eine kleine Fräse im Handstück geschoben, in den anderen ist die Drahtschlaufe eingelassen. Das Homogenisieren wird unter einer starken Lupe oder einem Präpariermikroskop vorgenommen, wobei das Handstück mit dem Homogenisator zweckmässigerweise an einem festen Stativ in der gewünschten Lage fixiert ist; auch die Gewebsprobe in der Kapillare kann mittels eines einfachen Halters auf dem Tisch des Präpariermikroskops festgehalten werden. Zur Herstellung des Homogenisators führt man ein ausreichend langes Drahtstück, zur Schlaufe gebogen, in einen dünnen Polyäthylenschlauch und verbindet beide miteinander entweder durch vorsichtiges Erwärmen oder mit etwas rasch härtendem Kitt. Mit einer Uhrmacherpinzette wird der Scheitel der Schlaufe zu einer feinen Spitze gebogen und die ganze Schlaufe dabei einmal verdrillt. Die hohe Elastizität des verwendeten Drahtes macht ein Zentrieren der einzelnen Teile überflüssig. Die beim Homogenisieren auftretende Wärme ist unbedeutend, wenn nicht mit zu hoher Geschwindigkeit gearbeitet wird. Das aufgearbeitete Material kann entweder in der Kapillare weiter verarbeitet oder verlustfrei aus dieser entfernt werden. An der Drahtschlinge haftende Materialteilchen werden durch die hohe Oberflächenspannung des Flüssigkeitsmeniskus der Kapillare in der Flüssigkeit zurückgehalten.

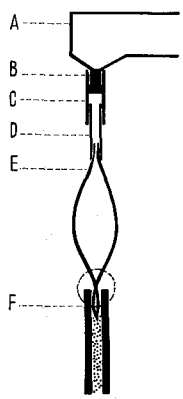


Fig. 1

Fig. 2

Fig. 1. Schematische Gesamtansicht des Mikro-Homogenisators. A = Handstück eines Zahnbohrers; B = Kleine Fräse; C = Polyäthylenschlauch; D = Polyäthylenschlauch; E = Drahtschlaufe; F = Kapillare mit Material.

Fig. 2. Vergrösserter Ausschnitt aus Figur 1 zeigt die Spitze der Drahtschlaufe und die Verdrillungsstelle. Die Spitze dient lediglich der leichteren Einführung des Gerätes in die Kapillare; das Homogenisieren erfolgt mit dem bauchigen Teil der Schleife oberhalb der Verdrillungsstelle.

Einzelheiten der Konstruktion sind der Figur zu entnehmen. Draht: Nikrothal L, 60 oder 35 $\mu$m Durchmesser; AB Kanthal, Halsthammar, Schweden. Kapillare: Microcap disposable pipettes; Drummon Scientific Company, Broomall, Pa. USA.

*Summary.* Homogenation of very small amounts of biological material (e.g. single cells) can be carried out without losses by using a loop of stainless steel thread inside of a capillary. A useful device is described.

D. EICHNER

*Anatomisches Institut der Universität Münster (Westfalen, Deutschland), 5. Mai 1966.*

PRO EXPERIMENTIS

## A Vital Stain for the Specialized Tissues of the Heart

Despite the large number of histological studies on the so-called specialized tissues of the mammalian heart[1,2], it would appear that no specific stain has been used which would show up these tissues as distinct from the myocardium. However, HOLMES[3], using a technique described by MITCHELL[4] for the study of nerve endings, observed that some myocardial fibres were stained by methylene blue. This observation has apparently not been pursued.

A stain specific to the specialized tissues would be useful in the localization of specialized cells and their conduction pathways, and in confirming the identification of cells for study by microelectrode techniques or by electron microscopy. Thus in the present study the observation of HOLMES has been followed up with a view to ascertaining the specificity and reliability of the use of methylene blue for this purpose. In addition, since methylene blue is

[1] J. S. ROBB and R. PETRI, in *The Specialized Tissues of the Heart*, Symposium, Rio de Janerio 1960 (Ed. A. PAES DE CARVALHO, W. C. DE MELLO, and B. F. HOFFMAN; Elsevier, Amsterdam-London-New York).

[2] R. C. TRUEX, in *The Specialized Tissues of the Heart*, Symposium, Rio de Janeiro 1960 (Ed. A. PAES DE CARVALHO, W. C. DE MELLO and B. F. HOFFMAN; Elsevier, Amsterdam-London-New York), p. 22.

[3] R. L. HOLMES, J. Anat. *91*, 259 (1957).

[4] G. A. G. MITCHELL, Acta anat. *18*, 81 (1953).

described as a non-toxic stain[5] and has been administered vitally[3,4,6], it was hoped that it would seriously modify neither the behaviour nor the ultrastructure of cells receiving the stain, so that these properties might be usefully studied after staining.

The hearts of 25 rabbits were used in this study. The rabbits were anaesthetized with nembutal and the hearts excised. The right side of each heart was opened and stained in 0.0025% solution of methylene blue in oxygenated Tyrode at 30 °C for periods of from 5–30 min. In a few experiments transmembrane potentials were then recorded using microelectrodes. Often the whole of the right atrium was fixed and mounted, using the technique of MITCHELL[4] slightly modified (the specimens were left in ammonium picrate for from 30 min to 2 h and in a glycerine jelly/picrate mixture for from 30 min to 2 h, and were then ready for mounting and viewing). In other experiments, selected areas of stained tissue were prepared as above, or were fixed in glutaraldehyde and embedded in methacrylate or araldite for electron microscopy.

In all rabbits studied, clearly defined bundles or groups of fibres were stained and in their general features the results showed a high degree of consistency. The structures of many cells were clearly outlined, as were some atrial pathways. For histological studies, 20–30 min has proved to be the optimum staining period. For identification of fibres prior to electrophysiologic or electron microscopic studies, shorter staining periods were sufficient.

Figure 1 is a schematic diagram of stained tissues found in the right atrium of every rabbit studied. It can be seen that the locations of many of the stained fibres were in approximation to the locations of characteristic action potentials observed by PAES DE CARVALHO[7]. In particular, distinctive arrays of stained fibres were observed where PAES DE CARVALHO located the pacemaker and the atrio-ventricular node (AVN) where the cardiac activity is delayed prior to its spread into the ventricles. Bundles of fibres which correspond to what PAES DE CARVALHO termed 'the sino-atrial ring bundles' (SARB) were especially sensitive to the stain, while fibres in the atrio-ventricular ring also appeared sensitive to it. A more detailed description and discussion of results will be given elsewhere.

After staining, the right atrium was regularly found to maintain activity for periods up to 4 h. Action potentials were recorded from the stained SARB and AVN and exhibited the expected shape, though their amplitudes may have been slightly attenuated. Stained cells examined by electron microscopy suffered no apparent structural damage if fixed within 30 min of staining. Cells fixed 2 h after staining had occasionally an abnormal appearance, but this may have represented deterioration due to the time delay rather than the effect of the methylene blue.

Work is proceeding on further study of the areas already known to have specialized function, and on further study of the other stained tissues, with a view to establishing whether or not they too have specialized function.

Methylene blue has been observed to stain some fibres in preference to others. Many of these were apparently fibres with known specialized function. Greater exposure of the specialized tissue may have contributed to their greater acceptance of the stain. In any case, the stain aided the study of these tissues. The stained fibres have not been observed to suffer modification in their behaviour or ultrastructure. Thus methylene blue appears to be a useful means of localizing specialized fibres and of correlating histological and electrophysiological findings[8].

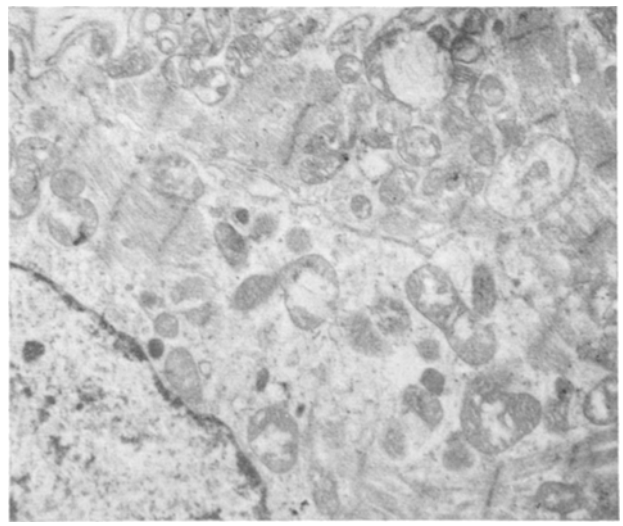

Fig. 2. Electron micrograph of tissue from the atrio-ventricular node. The tissue was stained with methylene blue and fixed in glutaraldehyde. × 7500.

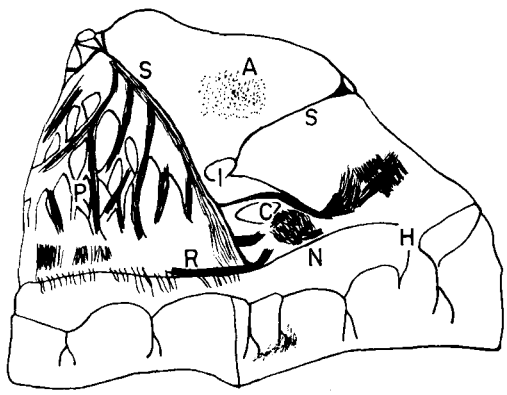


Fig. 1. Schematic diagram of the right atrium of the rabbit, opened after PAES DE CARVALHO[7]. Tissues most sensitive to methylene blue staining are illustrated. A = Sino-atrial node; C = Coronary sinus; H = His bundle; I = Inferior vena cava; N = Atrio-ventricular node; P = Pectinate muscle; R = Atrio-ventricular ring; S = Sino-atrial ring bundle.

*Résumé.* On constate que certaines fibres sous l'action du méthylène bleu présentent une coloration accentuée. Une proportion importante de ces fibres ont des fonctions apparemment spécialisées. Les fibres teintes ont gardé apparemment leur ultrastructure et leur fonction.

J. W. EMBERSON and C. E. CHALLICE

*Department of Physics, University of Alberta at Calgary, Calgary (Alberta, Canada),*
*March 23, 1966.*

[5] H. J. CONN, in *Biological Stains* (Williams and Wilkins, Baltimore 1961), p. 101.
[6] M. R. MILLER and M. KASAHARA, Am. J. Anat. *115*, 217 (1964).
[7] A. PAES DE CARVALHO, in *The Specialized Tissues of the Heart*, Symposium, Rio de Janeiro 1960 (Ed. A. PAES DE CARVALHO, W. C. MELLO, and B. F. HOFFMAN; Elsevier, Amsterdam-London-New York), p. 115.
[8] This research was aided by a grant from the National Research Council of Canada.