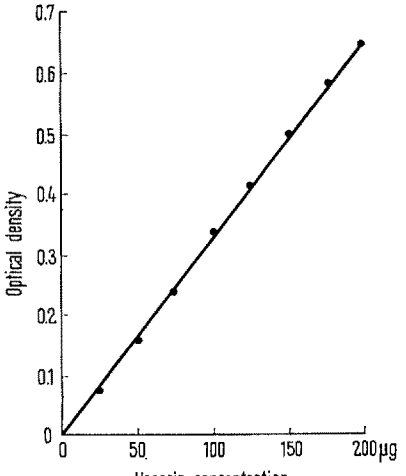
bath at 37°C for 60 min. The resulting turbidity as measured by optical density was determined with a Beckman DU spectrophotometer against the blank at 500 mμ with a slit of 10 mm.

When the standard AMPS were added to the prescribed NaCl-CPC solution, it was found that (1) only heparin produced turbidity by the formation of heparin-CPC complex, whereas no turbidity was produced by hyaluronic acid, heparitin sulfate and chondroitin sulfates, and (2) within the range of heparin concentration studied, optical density of the heparin-CPC complex was proportional to the concentration of heparin (Figure). When 100 μg of hyaluronic acid, heparitin sulfate and chondroitin sulfates were added individually or collectively to 100 μg of heparin solution, the recoveries of heparin added to these AMPS ranged from 98 to 100%.

This procedure was applied to the estimation of heparin contents in aortic tissue; the AMPS extracted from human and bovine aortae were prepared in acetate buffer solutions and examined in the manner described above. The bovine aortic AMPS gave an average heparin yield of 0.75 mg (ranging from 0.4 to 1.3 mg) per 100 g of starting wet tissue weight. No detectable turbidity resulted, however, with any human aortic AMPS, even though amounts equivalent in weight to those of the examined bovine aortic AMPS were utilized.



Relation between the heparin concentration and the turbidity.

To the author's knowledge, a method for direct estimation of heparin which distinguishes heparin from other AMPS has not yet been reported. The present procedure is selective for heparin and has the advantage that the heparin is readily recoverable from the heparin-CPC complex and is available for further analyses; heparin is dissociated from the heparin-CPC complex by increasing the concentration of NaCl to 2.1 *M* or greater. Analyses of the recovered samples showed approximately 32% uronic acid[12] and 24% hexosamine[13]; paper chromatography of the hexosamine[14] indicated it to be glucosamine. Insufficient material prevented further characterization.

In the last decade much attention has been called to the relationship between mast cells and AMPS in arterial walls, especially in respect to arteriosclerosis and the potent physiological functions of heparin and related substances as anticoagulant compounds and as stimulators for lipid clearing factor[15,16]. As it is evident that mast cells which produce heparin[17] are widely distributed in blood vessels of various species[7], it is of interest to ascertain whether and to what extent heparin occurs in aortic walls. Present observation would indicate that appreciable traces of heparin or related substances are present in bovine aortae but not in human aortae. This result would support the early reports of JORPES et al.[7] (1) that bovine aortic walls contain relatively more mast cells than aortic walls of other species, and (2) that crude heparin has been isolated from bovine aortae.

Zusammenfassung. Es wird eine vereinfachte Methode zur quantitativen Bestimmung des Heparins, bei Ausschliessung anderer Mucopolysaccharide, sowie Hyaluronsäure, Chondroitinschwefelsäure und Heparitinschwefelsäure, beschrieben. Es ergibt sich, dass das Aortagewebe des Rindes in kleinen Mengen Heparin enthält, während es in der menschlichen Aorta nicht gefunden werden konnte.

K. MURATA[18]

Department of Medicine, Loma Linda University School of Medicine, Los Angeles (California USA), May 18, 1964.

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PRO LABORATORIO

Apparatus for *in vitro* Studies of Mammalian Peripheral Nerves and Spinal Funiculi

In the apparatus developed by LEHMANN[1], and in its modification by RUDIN and EISENMAN[2], the temperature was controlled by immersing a watertight nerve chamber in a water bath. For the drainage of shunting fluids at the electrodes, the chamber and the input and output tubing for the gases had to be taken out of the bath and the chamber had to be opened. This type of apparatus was obviously inconvenient to handle, and drainage led to a temporary disturbance of the equilibrium in the nerve chamber.

It was found possible to overcome these disadvantages by constructing the apparatus illustrated in Figures 1 and 2, which consists of a system for moistening and warming

[1] J. E. LEHMANN, Am. J. Physiol. *118*, 600 (1937).
[2] D. O. RUDIN and G. EISENMAN, J. gen. Physiol. *37*, 505 (1954).

the gases (I) and a chamber with a lower (II) and an upper part (III). It is constructed mainly of perspex. The hollow spaces in the walls of the three parts are serially connected to a thermostat (Ultra-Thermostat, Original Lauda Type U3, Dr. R. Wobser KG., Messgeräte-Werk, Lauda/Tauber, Western Germany). Complete perfusion of the hollow spaces is effected by the insets No. 1 and No. 2 in parts II and III, respectively, and by a suitable arrangement of inlet and outlet pipes. The volumes of the solutions in parts I and II can be adjusted so as to provide the same volume to surface ratio (surface in contact with the walls), which is of special value in experiments concerned with temperature changes. The flow of gas is controlled with a gas-flow meter which has a measuring range of 1.6–16 l/h (KROHNE Type DK 26/N, Krohne, Duisberg, Western Germany). To disperse the gas into fine bubbles, it is passed through a fritted glass disc (3) with a pore diameter of 10–20 μ (P_4, Pyrex S.A., Paris). Ascending the column of 0.9% saline with a vapour pressure similar to that of Krebs' and Hastings' solution, the bubbles are humidified and warmed. In tube No. 4, which is filled with glass cylinders having an internal diameter of 4 mm, an external diameter of 5 mm, and a length of 5 mm, the gas is removed from the small drops resulting from the bursting of the bubbles. A needle connected with the outlet pipe of tube No. 4 conducts the gas to the bottom of part II, where the bubbles provide for continuous mixing of the solution. The gases leave the chamber through two holes (5). The temperature of the gas phase is measured with a needle applicator (Type K8) inserted into the chamber through hole No. 6 and connected to a universal electric thermometer (Type TE3, Ellab A/S, Copenhagen, Denmark).

The exchangeable electrodes in part III are swivel-mounted. Figure 2 shows the arrangement used for the experiment illustrated in Figure 3. Ag-AgCl electrodes with a diameter of 0.7 mm and isolated – except at the end – with heated Araldite coating resin 985 E (CIBA Ltd., Basel, Switzerland) are used for stimulation and recording. Completely isolated silver wires of the same diameter (S) serve to support the tissues. The electrodes are soldered to flexible isolated cables which leave the chamber through holes Nos 7 and 8. The soldered joints are watertightly fixed inside the perspex bar with Araldite adhesive resin (CIBA Ltd., Basel, Switzerland). Interference from bursting bubbles has never been observed. After washing of the tissues in the bath and after local application of solutions at the cathode or at a recording electrode, the shunting fluids have to be drained by capillarity. A suitably shaped hole in the solid ring of part III makes this possible without opening the nerve chamber (not shown in Figures 1 and 2).

Two tubes (9) are inserted into part III for the local application of solutions to tissues in the gas phase.

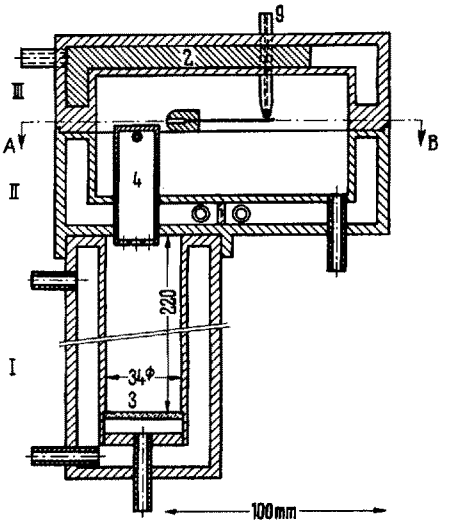

Fig. 1. Side-view. For explanation see text.

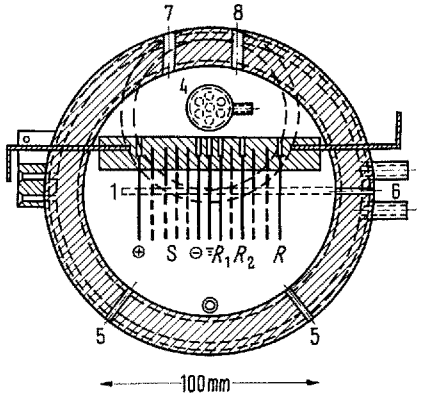

Fig. 2. Cross-section corresponding to A–B in Figure 1 rotated 90° to the right. Part I of Figure 1 is omitted here. For explanation see text.

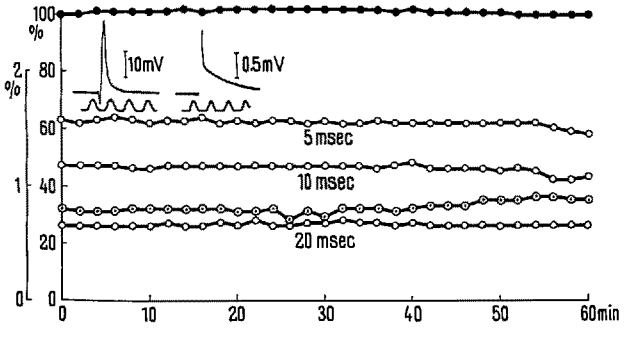

Fig. 3. Ventral funiculus of the thoracic spinal cord (cat), equilibrated in Hastings' solution, in the gas phase throughout (95% O_2 and 5% CO_2, 0.27 l/min, 37°C). Stimulation for recordings only. ●—● Supramaximal spike height in % of the value at 0 min (right ordinate). ○—○ Submaximal spike height in % of supramaximal spike height (right ordinate). o—o Supramaximal negative after-potential height at 5, 10, and 20 msec in % of supramaximal spike height (left ordinate). Left inset: supramaximal spike potential recorded monophasically at 1 cm of conduction distance (time scale 1000 cycles/sec); right inset: higher gain and slower sweep speed to show the negative after-potential (time scale 100 cycles/sec). Spike potential and negative after-potential retouched. Note stability of physiological properties during 50 min.

The experiment illustrated in Figure 3 shows that an isolated ventral spinal funiculus displays stable physiological properties for 50 min in the gas phase of the nerve chamber (isolation of the funiculus as described by RUDIN and EISENMAN[2]).

Zusammenfassung. Es wird eine thermostatisierte Nervenkammer mit vorgeschalteter Einrichtung zur Befeuchtung und Erwärmung der Gase beschrieben. Drainage kurzschliessender Flüssigkeit von den Geweben ist ohne Einfluss auf das Gleichgewicht in der Nervenkammer. Isolierte Rückenmarksfunikel zeigen in der Gasphase während 50 min konstante elektrophysiologische Eigenschaften.

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Forschungslaboratorien der CIBA AG, Basel (Switzerland), May 22, 1964.

[3] *Acknowledgment.* We wish to thank Dr. H. MAJER, of the Pharmaceutical Department of CIBA Ltd., Basel, for his valuable contribution to the design of the apparatus, and Mr. H. D. PHILPS for help with the manuscript.

TERMINOLOGIA

The Nomenclature of Multiple Enzyme Forms

Since the original demonstration that some enzymes may exist in a number of different forms in the same species or the same tissue, a considerable literature has accumulated on the topic. There has been no unanimity in these papers on the method of identifying a particular form, as WIEME[1] has pointed out. In a later note, KING and THOMPSON[2] stated that the specific question of the numbering of isoenzymes which have been separated by electrophoresis had been referred to the Standing Committee on Enzymes and the International Commission of Editors of Biochemical Journals. The Enzyme Commission in its Report[3] made no recommendation about multiple enzyme forms; and the International Union of Biochemistry, when it dissolved the Enzyme Commission and set up the Standing Committee on Enzymes[4], also set up a Sub-committee on Isoenzymes composed of the late E. J. KING, C. L. MARKERT, R. J. WIEME, F. WROBLEWSKI, and E. C. WEBB. After the death of E. J. KING, N. O. KAPLAN was appointed to the Sub-committee by the Bureau of I.U.B. This Sub-committee reached certain decisions which have been approved by the Standing Committee on Enzymes and are set out below.

Multiple enzyme forms may be distinguished from one another by any of several means, e.g. electrophoresis, chromatography, salt fractionation, ultracentrifugation, immunochemistry and reaction kinetics. The electrophoretic method has been most commonly employed, particularly in clinical laboratories, and numbering systems which have been employed have usually related to electrophoretic separation. Unfortunately, two quite different systems have been used. It is now recommended that:

'When multiple forms of an enzyme are identified by electrophoretic separation, they should be given consecutive numbers, the form having the highest mobility towards the anode being numbered one.'

This system is in conformity with that universally used for the fractions obtained by electrophoresis of serum proteins.

Such numbering systems are probably to be regarded as temporary expedients until information is available about the chemical differences between the various forms. If the molecules of the different forms vary in the nature and arrangement of protein sub-units, a nomenclature should be used analogous to that which has been successfully used in the field of haemoglobin chemistry.

The Sub-committee also considered the question of a suitable word to be used to describe multiple enzyme forms. MARKERT and MØLLER[5] originally proposed 'the term *isozyme* to describe the different molecular forms in which proteins may exist with the same enzymatic specificity'. Since then the forms *iso-enzyme* or *isoenzyme* have also been widely used, and the term has been limited to multiple forms in a single species. The majority of the Sub-committee felt that the latter forms were preferable as being more logical and in line with such terms as *isotope*.

It is therefore recommended that:

'Multiple enzyme forms in a single species should be known as *isoenzymes*, although since either form is readily intelligible this recommendation is not to be interpreted as excluding the use of "isozyme" if any individual author prefers it.'

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Department of Biochemistry, University of Queensland, Brisbane (Australia), April 16, 1964.

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CORRIGENDUM

J. BOURDILLON: *Flow of a Solution into a Tube Filled with Solvent: Static Concentration and Flow Concentration of the Solute.* Exper. vol. XX, fasc. 8, p. 423 (1964). An error occurs in the very first line on page 424. It should read as follows:

'For $U - S \geq 0.5$, we have:'.