Lösung soll nicht filtriert werden, da der Farbstoff am Filterpapier zurückbleibt. Ein spontanes Austreten des Farbstoffes ins Gewebe findet bei dieser Konzentration nicht statt.

Der gewünschte Effekt wird dadurch erzielt, dass es an der Elektrodenspitze durch die Elektrophorese zur Konzentration von Methylenblau kommt (Konzentrationseffekt); während die Methylenblaulösung in der Glaskapillare hellblau ist, entsteht an der Mikroelektrodenspitze bei Stromdurchgang ein intensiv gefärbtes, dunkelblaues, scharf abgegrenztes, langsam wachsendes kugelförmiges Gebilde. Im getrockneten Quetschpräparat bleicht die Methylenblaumarkierung nicht mehr aus.

Der Aussendurchmesser unserer Mikroelektrodenspitze betrug im Durchschnitt 1 μ, ihr Widerstand lag zwischen 5 und 10 Megohm. Die Mikroelektrode wurde mit dem positiven Pol einer Gleichspannungsquelle verbunden (da Methylenblau als Kation vorliegt), der negative Pol lag im Gewebe. Wird während 10 sec ein Strom von 3 μA durchgeschickt (was einer Strommenge von 30 μcoul entspricht), so bildet sich im Gewebe ein Farbfleck von etwa 10 μ Durchmesser, der die Zellen in unmittelbarer Nähe der Mikroelektrodenspitze anfärbt. Liegt die Elektrodenspitze intrazellulär, färbt sich nur diese einzige Zelle an. Es empfiehlt sich, den Strom langsam von 0 auf 3 μA ansteigen zu lassen, da sich sonst Gasblasen bilden können, die die Mikroelektrodenspitze verstopfen. Man kann dies aber auch weitgehend dadurch vermeiden, dass man einen geringeren Strom (0,5 bis 1,5 μA) während mehrerer Minuten fliessen lässt. Die Grösse dieses Farbfleckes hängt dabei nur von der Strommenge (Coulombs, d.h. Amperesekunden) ab, aber nicht von der Grösse der Mikroelektrodenspitze, somit auch nicht von deren Widerstand. Nach der Entfernung der Mikroelektrode wird aus der Umgebung des Einstiches ein Gewebsblock von etwa 3 mm^3 ausgeschnitten und mit der Plastik-Quetschmethode weiter bearbeitet[15] (Figur 1 und 2).

Die Methode ist einfach und gut reproduzierbar. Die physikalischen Eigenschaften der Mikroelektrode ändern sich durch die Füllung mit KCl-Methylenblaulösung und die elektrophoretische Injektion des Farbstoffes grundsätzlich nicht. Das ist eben für elektrophysiologische Versuche sehr wichtig.

Es ist zu hoffen, dass es gelingen wird, mit dieser kombinierten Methode von ein und derselben Zelle sowohl elektrophysiologische als auch morphologische und zytochemische Daten zu erhalten.

Summary. A method of electrophoretical marking of individual neurons with methylene blue by means of a glass micropipette (used in electrophysiology) is described. In combination with a new histological crushing technique, cytochemical analysis of whole undamaged cells thus marked can be correlated with electrophysiological findings in the same neuron.

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Isolated Perfused Dog Spleen Method

In order to study postganglionic adrenergic mechanisms Thoenen et al.[1] described a method for perfusion of cat spleen in vitro. According to this method the isolated spleen, kept in a plethysmograph containing liquid paraffin, is perfused by means of a constant flow rate pump. Using this method, variations in neurotransmitter output may be correlated with changes in total spleen volume and in perfusion pressure.

A similar technique has been used by Hertting et al.[2,3] in the cat and by Farmer[4] in the dog.

In the present paper an isolated dog spleen perfusion method is described which may present some advantages as compared with previously described techniques.

Method. Mongrel dogs weighing 5–8 kg are anaesthetized with pentobarbitone, 30 mg/kg, i.v. The abdomen is opened through a midline incision and the intestines removed from mid-duodenum to rectum. The vascular connections between the spleen and the stomach, omentum and pancreas are cut between ligatures.

The coeliac artery and portal vein are dissected from the surrounding tissues. After heparinization (10 mg/kg of a 50 mg/ml heparine Roche solution i.v.) both blood vessels are ligated and cut. The spleen is removed and polyethylene catheters type PE 240 and PE 200, reinforced by short stainless steel tubes at the inside of their tip, are introduced into the artery and vein. The spleen is immediately placed in the plethysmograph and perfused at constant pressure with a modified Krebs-Ringer bicarbonate solution (NaCl 6.92 g – $NaHCO_3$ 2.10 g, KCl 0.35 g, $MgSO_4 \cdot 7H_2O$ 0.29 g, KH_2PO_4 0.16 g, $CaCl_2$ 0.28 g, glucose 1.15 g, ascorbic acid 25 g, diaminoethaneacetic acid disodium salt 10 mg/l). The pH of this solution is brought to 7.1 at 20 °C by means of HCl, to reach a final pH of 7.4 at 37 °C after saturation with carbogen (95% O_2 + 5% CO_2). After adjusting the venous outflow resistance at 4 cm H_2O, the arterial inflow pressure is regulated in order to obtain a perfusion flow of 20 ml/min. The perspex plethysmograph (Figure 1) consists of 2 parts: (a) the bottom part measuring 23 × 13 × 3 cm and containing the heating tubes, 2 electrode passthroughs, passthroughs for both arterial and venous catheters, and a tube which permits evacuation of the liquid accumulated

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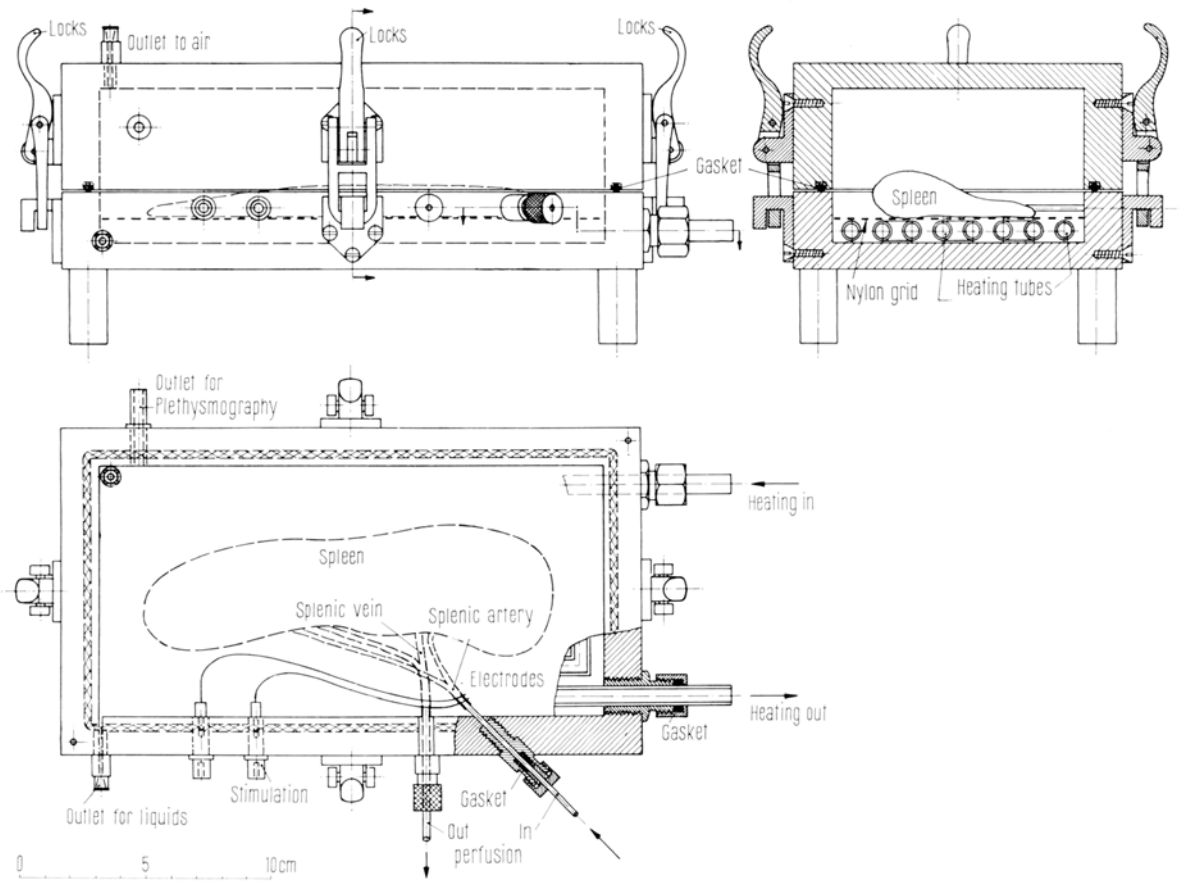

Fig. 1. Blue-print of the perspex plethysmograph.

at the bottom; the spleen lies on a nylon grid covering the heating tubes; (b) the top part measuring 23 × 13 × 5 cm and containing an outlet for the plethysmographic recording, an outlet with stop-cock leading to the outside air, and an opening for a thermometer; this part fits perfectly onto the bottom part, the plethysmograph being made air-tight by means of a rubber gasket and 4 locks.

Two Varian type G 22 recorders, equipped with A21A amplifiers, are used for registration.

Plethysmographic measurements are done by means of a small spirometer, the volume variations (up to 80 ml) of which are recorded by means of a displacement transducer.

The arterial in-flow and venous out-flow are measured by means of transistorized rotameters (Figure 2) with a range from 0–100 ml/min and a corresponding D.C. voltage output from 0 to ± 5 mV. Differential flow, corresponding to the difference in output voltages of both flowmeters, may also be recorded.

Splenic nerve stimulation is made for 10 sec by means of chlorurated silverwire electrodes (electrical impedance between the electrodes varies normally from 300–600 ohms) of 0.2 mm diameter, twisted around the sympathetic nerve fibres, which are adjacent to the splenic artery, using a Grass model S4B stimulator, delivering square waves of 2 msec duration at a frequency of 6/sec and a voltage of 5–15 V. This frequency of stimulation may be considered as being near to the maximal physiological values[5]. Using higher voltages, on the other hand, did not improve results but, on the contrary, damaged sympathetic nerve fibres.

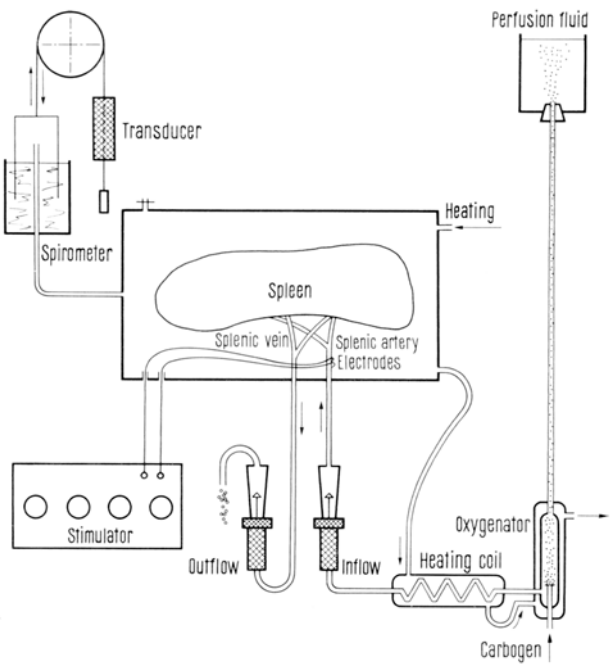

Fig. 2. Experimental set-up.

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The venous effluent is collected into a chilled vessel containing 0.2 ml $HClO_4$ (60%) per 10 ml during several collection periods of 90 sec each, before, during and after stimulation. After volumetric measurement, the effluent is centrifuged, eventually stored at $-12\,°C$, and assayed for its noradrenaline content using the spectrofluorometric method described by SHARMAN et al.[6].

Results. The following preliminary observations illustrate the applicability of the method.

Isolated dog spleens were perfused for 90 min without stimulation. Ten minutes after starting the perfusion the caudal third of the spleen was ligated and excised, deep-freezed and subsequently assayed for its noradrenaline content, which was compared with the content immediately after the end of the perfusion. Samples of venous effluent were taken every 15 min and their noradrenaline content assayed.

Under these experimental conditions, a spontaneous release of noradrenaline of 0.37 ± 0.08 ng/ml was observed. The noradrenaline content of the spleen amounted to 2.6 ± 0.4 µg/g, without any significant difference after 90 min of perfusion.

Experiments in which differential flow responses were studied in relation to varying frequencies and voltages of stimulation (Figure 3), showed the optimal stimulation frequency and suitable voltage to be 6/sec and 5 V respectively.

Using this type of stimulation the noradrenaline output varied between 1.1 and 2,1 ng/stimulus. After cocaine infusion (10 µg/min) the noradrenaline output varied between 3.3 and 4.4 ng/stimulus. This infusion of cocaine, resulting in a blockade of transmitter re-uptake, considerably increased the changes in differential flow and splenic volume after splenic nerve stimulation (Figure 4).

Infusion of phenoxybenzamine (20 µg/min), on the other hand, resulted in a blockade of both re-uptake and α-receptors[7], as shown by a markedly increased over-flow of noradrenaline in the splenic effluent and a complete absence of volume and flow changes after splenic nerve stimulation (Figure 5).

Infusion of a mixture of cocaine (10 µg/min) and phenoxybenzamine (20 µg/min) had a similar effect as after phenoxybenzamine alone.

These preliminary experimental results confirm previous observations reported by several authors[1,2,4,7].

It may be mentioned that measurements of in- and out-flow by means of rotameters have also proved to be useful in the course of isolated spleen perfusion experiments in which a constant flow rate is applied. In this set-up the in-put measurement provides a control of flow constancy, whereas the out-put measurement permits a permanent monitoring of changes in splenic flow[8].

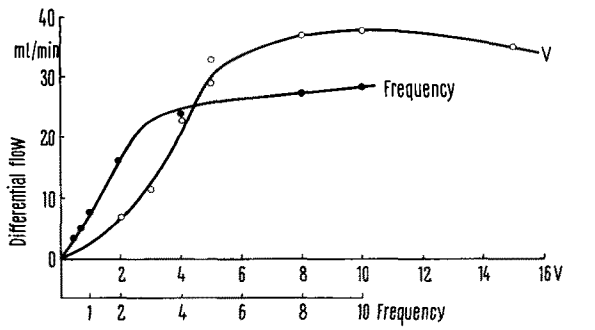

Fig. 3. Differential flow responses in relation to varying frequencies and voltages of stimulation.

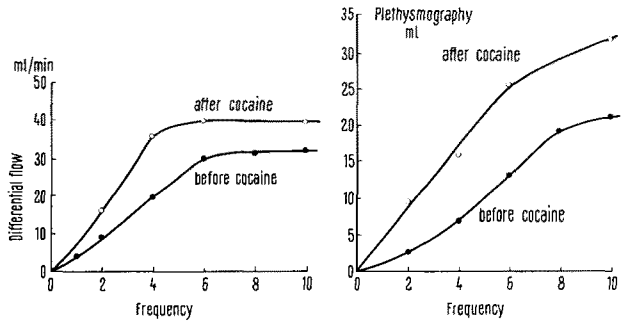

Fig. 4. Changes in differential flow and splenic volume before and after cocaine in relation to varying frequencies of stimulation.

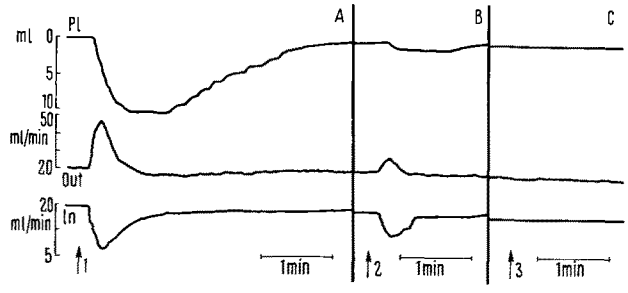

Fig. 5. Pl, plethysmogram; Out, outflow; In, inflow; ↑ 1, control stimulation; ↑ 2 and ↑ 3, stimulation after phenoxybenzamine; between A and B: interval of 10 min; between A and C: interval of 20 min.

Zusammenfassung. Eine Methode zur Perfusion der isolierten Milz des Hundes wird beschrieben, bei welcher die Variationen im Noradrenalin-output nach Sympathikusstimulation mit Veränderungen im In-flow, Out-flow und differentiellen Flow sowie im Milzvolumen korreliert erfasst werden können. Der Einfluss von Kokain und Phenoxybenzamin auf diese Paramcter wird geprüft.

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