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An apparatus for performing filtration assays in hyperbaric atmospheres

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Sauter JF, Wankowicz PG, Miller KW. An apparatus for performing filtration assays in hyperbaric atmospheres. Undersea Biomed Res 1980: 7(4):257–263.—A detailed understanding of the effects of diving gases on the central nervous system will require extensive neurochemical studies. One standard tool of the neurochemist is the filtration assay used to measure the binding of neuroeffectors to receptors, for example. We describe here a system for carrying our 12 such assays in hyperbaric gaseous environments. The device fits in a small pressure chamber and consists of a syringe drive for delivering solutions to the filter units brought in turn under an injection nozzle by a conveying carousel. A modified commercial filter apparatus is held on top of a vacuum-tight collection compartment. Solution is injected into an incubation well above the filter. After stirring and incubation, filtration is performed by allowing the positive pressure of the chamber to burst a diaphragm at the bottom of the incubation well and push the solution through the filter. The binding of [^{3}H]acetylcholine to receptor-rich membranes from *Torpedo californica* was studied. In one experiment a percentage receptor occupancy of 49.5 ± 1.4 at 5 atm was reduced to 41.7 ± 0.9 at 300 atm.

hyperbaric filtration apparatus
gas pressure
filtration assay

To obtain a complete understanding of the physiological effects of pressure and of diving gases will require a detailed approach in which the effects on intact animals are compared to the results from electrophysiological and neurochemical studies on isolated systems. Few neurochemical studies have been reported under high pressure of gases (1). One common tool used in neurochemical studies is the filtration assay, which may be used, for example, to measure binding of neuroeffectors to their receptors. In this note we describe a filtration apparatus for use in gaseous hyperbaric environments, and we demonstrate that the binding of [^{3}H]acetylcholine to its receptor may be measured with high precision in a small chamber pressurized to as much as 300 atm with helium.

GENERAL DESCRIPTION OF APPARATUS

Our overall objective was to design a system capable of achieving a dozen filtration assays during a given pressure exposure. We also needed to mix accurately known volumes of

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various solutions in situ and stir them before filtration. The solution had to be achieved within the confines of an existing pressure chamber (2) that provided a cylindrical working space of 18 cm i.d. by 61 cm.

A general view of the final apparatus is shown in Fig. 1. It consists of three parts: a syringe drive *(left)* for delivering solutions; a mixing and filtration carousel with 12 movable filtration units *(right)*; and a system for applying suction to the filter (not shown, but see Fig. 3).

The syringe drive subsystem is conventional and is not described in detail. Because of the space limits in our chamber this unit was custom made, but any commercial system (e.g., Harvard pump, Harvard Apparatus, Millis, MA; or Hamilton Microlab P, Whittier, CA) could be substituted. Two features of our system are of sufficient interest to deserve attention. Stepping motors, working on a threaded lead principle, were folded back on the syringe so that they pull, rather than push, the piston. This arrangement saves space. The system can deliver a series of small aliquots from a large syringe (typically 1.5 ml from a 50-ml syringe) with a precision better than 1%, as determined both on the bench and in the chamber by weighing aliquots of water delivered from a syringe. Plexiglas mounting plates were used to allow the operator an unobstructed view through the unit.

Filtration units

The filtration unit had to allow the solutions to be mixed, incubated, and subsequently filtered. Standard Millipore or Whatman glass fiber filters were to be used, and for simplicity these were to be held in a suitably modified and commercially available filter unit. The design now in use is illustrated in Fig. 2. It consists, from top to bottom, of four functional units: 1) an incubation well, 2) a breakable diaphragm, 3) a filter holder, and 4) collection vessel. The whole unit disassembles to allow both retrieval of filter and filtrate after the experiment and

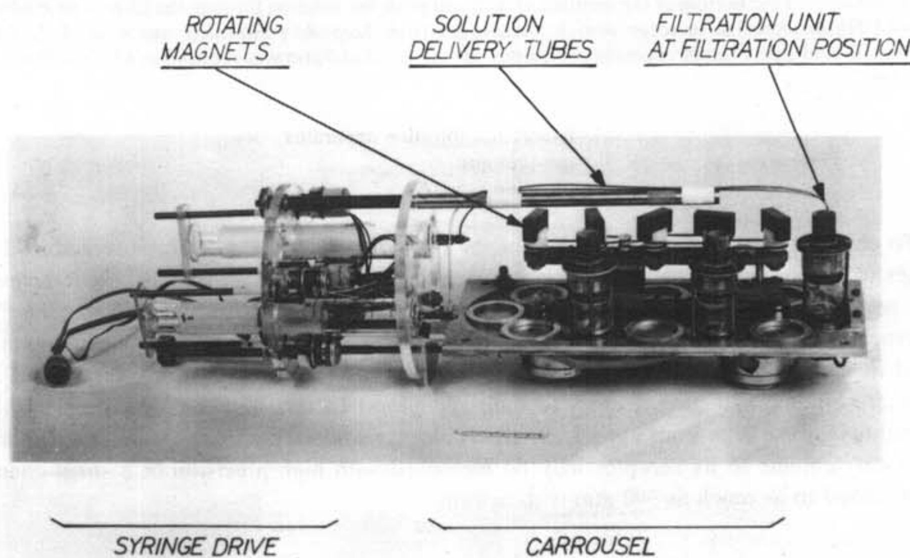

Fig. 1. View of the complete filtration system, showing the syringe drive with 2 of 4 syringes mounted, carousel with 3 of 12 filter units, and 5 rotatable bar magnets on top. Rings on the carousel are spacers used only in experiments with less than 12 filters.

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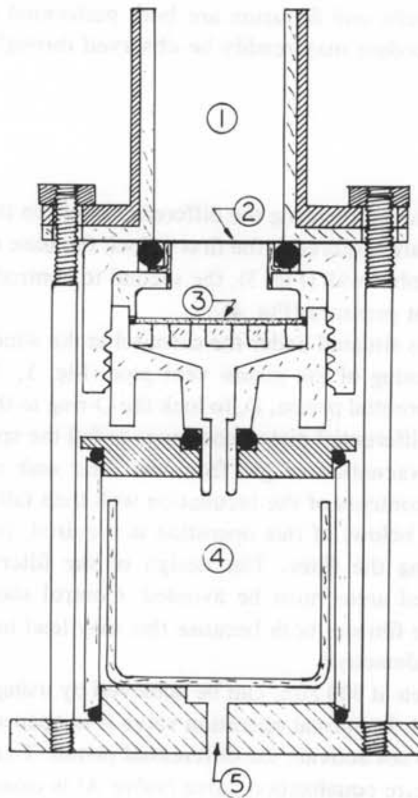

Fig. 2. Section of a filtration unit: 1, incubation well; 2, diaphragm; 3, filter; 4, collection vessel; 5, gas evacuation vent.

decontamination and washing before reuse. As many as12 such units may be accommodated on the carousel.

The incubation well is a Delrin-lined cylinder (1.5 cm i.d. by 3 cm) open at the top and closed at the bottom with a burstable plastic diaphragm (polyvinylchloride, 10–20 μm thick. Sandwich Bag, Purity Supreme, Boston, MA). Solutions may be added to this from the syringe drive subsystem and mixed by a Teflon-coated, magnetically driven stir bar. The filter holder is a Nuclepore (Pleasanton, CA) in-line type, 25 mm, modified externally to seat between the diaphragm and collection vessels on O-rings. The filtrate drains into the lower Plexiglas-walled chamber, where it is collected in a 5-ml glass vessel. A central hole in the base of the unit provides a pressure vent.

Filtration carousel

Twelve filtration units can be positioned in the carousel's raceway. They may be driven around the raceway by two motor-driven capstans driving a compressible O-ring belt (Buna N, 70 durometer). The belt constitutes the inner race and drives the filter units by rotating them against the outer stationary race. The central division also contains a variable speed 12-V DC motor linked by belt drive to five axially mounted horizontal bar magnets, which may be rotated to drive the stirrers in the incubation wells.

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Filling of the incubation wells and filtration are both performed at the end opposite the syringe drive, where the procedure may readily be observed through a chamber window.

Suction system

Suction for filtration is achieved by using the difference between the chamber pressure and room pressure. Two devices are required—the first to lock the base of the filtration units to a pipe venting through the chamber wall (Fig. 3), the second to control the pressure differential between the pipe and ambient pressure (Fig. 4).

The filtration lock (Fig. 3) is situated under the carousel at the window end position (Fig. 1, *right*). It is activated by opening of the piston vent pipe (Fig. 3, A) to low pressure, thus automatically raising the differential piston, D, to lock the O-ring to the base of a filtration unit at B. As the pressure in the differential piston continues to fall the spring-loaded check valve, E, opens; the subsequent evacuation of gas from the filter unit results in rupture of the diaphragm (Fig. 2), and the contents of the incubation well then fall onto, and pass through, the filter. Close control (see below) of this operation is required, particularly at the highest pressures, to avoid rupturing the filter. The design of the filter support screen is also important—large unsupported areas must be avoided. Control should be such as to avoid dispersing or aerosolizing the filtrate, both because this may lead to experimental anomalies and because the filtrate is radioactive.

Very smooth filtration, even at 300 atm, can be achieved by using the exhaust control unit shown schematically in Fig. 4. In normal operation valve A is open so that any minor leaks in the closed valves B and C do not activate the differential piston. To achieve filtration valve B is opened and then the pressure equalization valve (valve A) is closed. Valve C is controlled automatically because this enables achievement of the highest reproducibility, but manual control is satisfactory; we use a Kim-1 (Commodore, Santa Clara, CA) microprocessor for this, but any adjustable timing device or circuit would be suitable. Opening C allows venting to the room through the micrometer valve D and activates the filtration sequence described above. The setting of the micrometer valve is determined empirically, starting with a small setting and increasing the setting until the desired result is achieved. For this purpose it is very useful to be able to observe the filtration process through the chamber window. In practice two such valves are required to give a wide enough range of flows to achieve filtration from 2 atm to 300 atm. At any given pressure a range of micrometer settings will be found. At the lowest setting a slow filtration sequence, requiring about 2 s to break the diaphragm and 5 s to filter, yields very smooth filtering. At the highest setting almost instantaneous filtration occurs, but gas flow must be stopped quickly to prevent splattering the contents of the collection vessel. After filtration, B is closed, and A is opened to ensure the return of the suction piston to its lower position. Because the filtrate is radioactive it is important to effectively baffle the exhaust pipe E to prevent aerosolized radioactivity from entering the laboratory during vigorous filtration. For this purpose we lead the exhaust pipe to the bottom of a plastic bottle loosely filled with absorbent paper.

One further parameter that is conveninet to fine tune is the dead volume between C and D. This space is initially at 1 atm when valve C is opened; the resulting pressure surge is ideally sufficient to raise the differential piston but not to open the check valve. The latter opens as pressure falls under control of needle valve D. We adopted this pneumatic method because of space limitations, but if these are not critical, electrical elevation of a simpler lockup for delivering suction to the filter could well be satisfactory.

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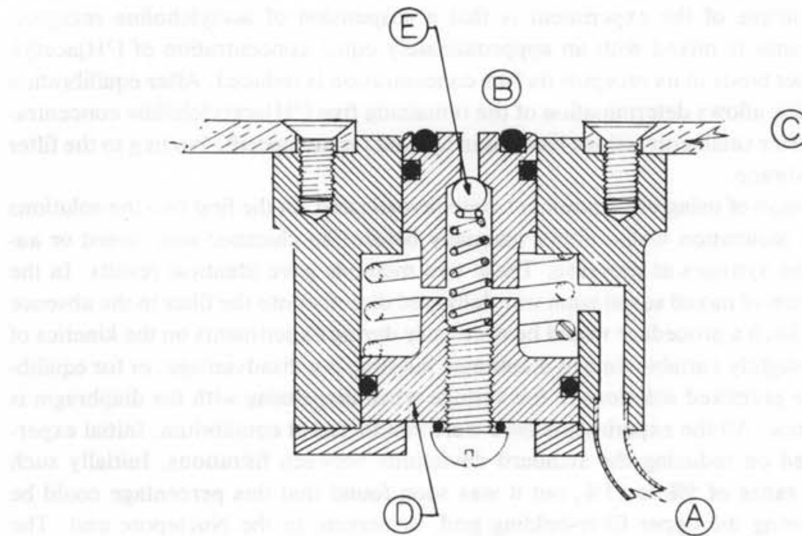

Fig. 3. Cross section of the lock that connects filtration units to suction during filtration. A, piston vent pipe connecting to controls (see Fig. 4) outside the chamber; B, top of differential piston that seals to the gas evacuation vent on the bottom of a filtration unit; C, level of the carousel table; D, differential piston that moves upward upon evacuation through A; E, spring-loaded check valve that opens after B locks to the filtration unit.

PROCEDURES AND RESULTS

The system used to test and develop our methodology was the binding of [^{3}H]acetylcholine to receptor-rich membranes isolated from the electroplaque of the ray *Torpedo californica*. Experimental procedures given here are only concerned with the high pressure aspect of the experiment. The techniques used are well established and details may be found elsewhere (3,

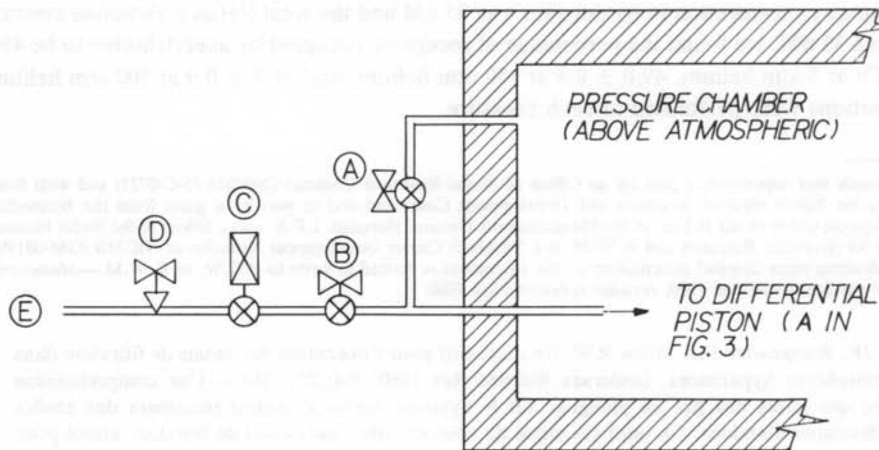

Fig. 4. Schematic of the external pneumatic arrangement used to control filtration. Valve C is electrically activated, valve D is a micrometer needle valve used to adjust the speed of filtration. System is duplicated for low pressure filtration (see text).

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4). Briefly the principle of the experiment is that a suspension of acetylcholine receptor-containing membranes is mixed with an appproximately equal concentration of [^{3}H]acetylcholine. As the latter binds to its receptor its free concentration is reduced. After equilibration is complete, filtration allows determination of the remaining free [^{3}H]acetylcholine concentration in the filtrate after small corrections have been applied for nonspecific binding to the filter and to the biomembrane.

Three different ways of using our equipment were investigated. In the first two the solutions were added to the incubation well—either manually before the chamber was closed or automatically from the syringes at pressure. These two methods gave identical results. In the third method a stream of mixed suspension was delivered directly onto the filter in the absence of any diaphragm. Such a procedure would be necessary during experiments on the kinetics of binding, when the slightly variable time of diaphragm bursting is a disadvantage, or for equilibrium studies on the premixed solutions in the syringe when dispensing with the diaphragm is merely a convenience. All the experiments here were conducted at equilibrium. Initial experiments concentrated on reducing the standard deviations between filtrations. Initially such errors were in the range of 5% to 15%, but it was soon found that this percentage could be improved by removing the upper filter-holding grid, or screen, in the Nuclepore unit. The latter was designed to allow bidirectional flow and had been retained to avoid filter movement during compression. It is not shown in Fig. 2. With the screen removed the diaphragm method consistently gave standard deviations of about 2% in groups of four filtrations. Comparable accuracy could only be achieved in the absence of the diaphragms when suction was commenced a few seconds after delivery of the solution to the filter. Simultaneous suction and filtration at high pressure increased the standard deviation to 8%–10% at 300 atm.

The best standard deviation achieved above is comparable to what may be achieved by using conventional equipment on the bench. Such low variability suggested that no receptor-containing membranes were being forced through our filters. This conclusion was confirmed by refiltering on the bench filtrates collected from high pressure filtrations. During the early experiments catastrophic failure of the filters occurred sometimes, but development of the technique of applying suction, described above, has totally prevented this.

As an example of the sort of results that may be obtained, in one experiment in which the total receptor concentration in suspension was 35 nM and the total [^{3}H]acetylcholine concentration was 35 nM, we found the percentage of receptors occupied by acetylcholine to be 49.5 $\pm$ 1.4 (SD) at 5 atm helium, 49.0 $\pm$ 0.8 at 150 atm helium, and 41.7 $\pm$ 0.9 at 300 atm helium. Four filtrations were performed at each pressure.

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Anyone desiring more detailed information on the equipment is invited to write to P.G.W. or K.W.M.—*Manuscript received for publication March 1980; revision received May 1980.*

Sauter JF, Wankowicz PG, Miller KW. Un dispositif pour l'exécution des essais de filtration dans les atmosphères hyperbares. Undersea Biomed Res 1980: 7(4):257–263.—Une compréhension détaillée des effets des gaz de plongeur sur le système nerveux central réclamera des études neurochimiques étendues. Un outil classique du neurochimiste est l'essai de filtration utilisé pour mésurer l'agrégation des effeteurs neuraux aux récepteurs par exemple. Nous décrivons ici un système pour mettre à exécution notre douze essais dans les environnements hyperbares gazeuses. Le dispositif s'adapte à une petite chambre de pression et consiste en une attaque-seringue pour délivrer des solutions aux unités filtres amenés en tour sous un ajutage d'injection par un carousel porteur. Un dispositif filtre commercial modifié est tenu sur le sommet d'un compartiment de

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rassemblement vacuum-étanche. De la solution est injectée dans un puits d'incubation au-dessous du filtre. Après de l'agitation et de l'incubation, la filtration est exécutée permettant la pression positive de la chambre d' éclater un diaphragme sur le fond du puits d'incubation et pousse la solution à travers le filtre. L'agrégation des membranes riches de recepteurs [^{3}H]acétylcholine du *Torpedo californica* a été studiée. Dans une épreuve une occupation recepteur pourcentage de 49.5 ± 1.4 à 5 atm a été à 41.7 ± 0.9 à 300 atm.

dispositif de filtration hyperbare
préssion de gaz
essai de filtration

REFERENCES

1. Fish E, Shankaran R, Hsia JC. High pressure neurological syndrome: antagonistic effects of helium pressure and inhalation anesthetics on the dopamine-sensitive cyclic AMP response. Undersea Biomed Res 1979; 6:189–196.
2. Miller KW, Wilson M. The pressure reversal of a variety of anesthetic agents in mice. Anesthesiology 1978; 48:104–110.
3. Cohen JB, Changeux JP. The cholinergic receptor protein in its membrane environment. Annu Rev Pharmacol 1975; 15:83–103.
4. Sauter JF, Braswell LM, Miller KW. Action of anesthetics and high pressure on cholinergic membranes. *In:* Fink BR, ed. Progress in anesthesiology, Vol. 2. New York: Raven Press, 1980: 199–207.

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