ing beneath them, and in fact a branch of the nerve seems to actually pierce the dermis right at the point[8]. In any case I should like to urge that the presence of cutaneous nerves beneath so many acupuncture points not be dismissed too hastily at this point in our present knowledge (or lack of it) of the mechanisms of acupuncture.

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Note added by S. Weidmann, Berne: The report by Chiang et al.[2] seems to be undisputed as an experimental fact. However, from the way I quoted this work[1] readers might indeed have got the impression that acupuncture points in relationship with cutaneous nerves do not exist. The present contribution by an anatomist is taken as a valuable comment to show that the afferent pathways taken by acupuncture signals are incompletely understood, as recently re-stated by Chang[9].

PRO EXPERIMENTIS

Tangential flow filtration of *Bordetella pertussis* submerse cultures

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Summary. A procedure is reported for the large scale separation of *Bordetella pertussis* microorganisms from liquid culture media by tangential flow filtration (cross flow filtration) using anisotropic membranes with a cut-off limit of 1×10^6 daltons, and microporous membranes with a pore size of 0.22 μm.

The large scale separation of microorganisms from culture broth in vaccine preparation is generally achieved using methods that require complex and very expensive apparatus (continuous flow centrifugation, special filtration units with filter aids) or which are very time-consuming with consequently increased risks of contamination (repeated batch centrifugation).

Filtration techniques with a flow parallel to the filtering surface offer a new approach to the separation of solids from the liquid phase, and may also be applied to bacterial suspensions[1]. A tangential flow filtration system, which presents some particular advantages, has been used successfully in this laboratory for the concentration and purification of influenza viruses, using anisotropic membranes with a high molecular weight cut-off[2].

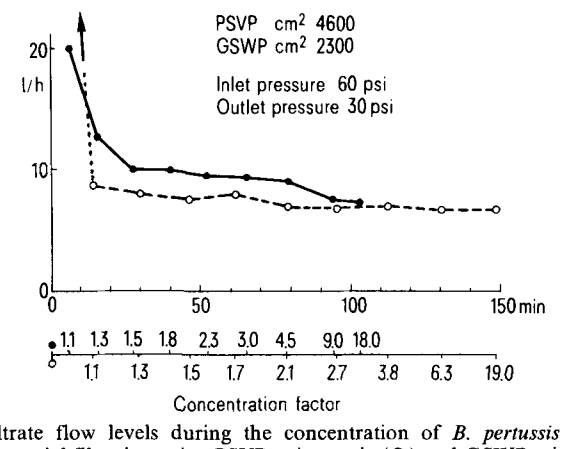
In this paper we report the results obtained using this system with 2 different types of membranes, for the collection and large-scale concentration of *Bordetella pertussis* cultures: the centrifugation technique was adopted for comparative purposes.

Results and discussion. These studies were performed on the Pellicon Cassette® System equipped with a piston pump, using anisotropic PSVP molecular filtration membranes with a cut-off limit of 1×10^6 daltons and a surface area of 4600 cm^2, or 0.22 μm GSWP microporous membranes with a surface area of 2300 cm^2 (Millipore, Bedford, Mass., USA). Molecular filtration membranes consist of a relatively dense skin 0.02–0.03 μm thick (the actual filtrating part), supported by a porous substructure about 150 μm thick, and this asymmetry, in contrast with the homogeneous structure of microporous filters, confers an anisotropic configuration to the membrane; because of these differences in design, the performance of the 2 types of membranes is quite different.

Batches of *B. pertussis* strain 509 submerse cultures, each composed of about 20 l, were concentrated to 1 l by forced tangential flow through the membranes, or were processed using the Sorvall mod. RC-3 centrifuge. The yield of the process was determined by the Opacity Units method (OU)[3], and the quality of the retentate and filtrate was evaluated by the Mouse Toxicity Test (MTT)[4]. The readings were taken, as shown in the table, using previously established levels of toxicity.

The figure shows a typical performance of the membranes during the concentration phase at constant pressure.

The flow levels of the filtrate per unit of time and surface area, obtained in these tests, appear to be higher when employing the microporous membranes; mean values of 0.527 ml $min^{-1} cm^{-2}$ were obtained with the GSWP type, as opposed to 0.0359 ml $min^{-1} cm^{-2}$ with the PSVP type.



Filtrate flow levels during the concentration of *B. pertussis* by tangential filtration using PSVP anisotropic (●) and GSWP microporous (○) membranes. Starting volumes: 18 l for PSVP membranes and 19 l for GSWP membranes. The high initial flow (not estimated) and the rapid passage to a steady flow rate in the case of the microporous membranes, are indicated by the arrow and the dotted line.

Lot 1326 II				
Centrifuge (16 l)			PSVP Membrane[c] (18 l)	
Pellet			Concentrate[d]	
Yield 65.6% (O.U.)[a]			Yield 88.9% (O.U.)	
M.T.T.[b]: −			M.T.T.: + +	
Lot 1338 I				
Centrifuge (16.5 l)			GSWP Membrane[e] (19 l)	
Supernatant	Pellet		Filtrate	Concentrate[d]
M.T.T.: + +	Yield 66.8%		M.T.T.: −	Yield 85.7%
↓	M.T.T.: −		↓	M.T.T.: +
PSVP Membrane			PSVP Membrane	
8× Concentrate			8× Concentrate	
M.T.T.: + +			M.T.T.: +	

[a] Opacity Units[3]; [b] Mouse Toxicity Test[4]. Levels of toxicity: (+ +) High mortality, (+) Low mortality, (−) Not toxic; [c] Anisotropic molecular filtration membrane: nominal molecular weight 1×10^6 daltons; [d] Concentrate washed with 10 l of phosphate buffer; [e] Isotropic microporous membrane: pore size 0.22 µm.

There is an evident difference in behaviour at the beginning of the concentration step: the anisotropic membranes tend to reach a steady flow level by gradually halving the rate, while the microporous membranes are characterized by a very high initial flow which is difficult to estimate because there is a very rapid decrease to the steady flow level (arrow and dotted line in the figure). In all the tests performed, it was surprisingly easy to obtain concentration factors of 20–30 times, especially using GSWP membranes; at higher concentrations, the dense bacterial suspension did not appear to clog the membranes unduly, but forced us to reduce the recirculation rate, thus also reducing the filtrate flow. The table shows the comparative data of the controls performed on 2 representative lots. The yield, in terms of Opacity Units, was always more favourable in the case of the membranes whose filtrate, unlike the supernatant obtained after centrifugation, was completely free of germs.

However, while the centrifugation technique allows the separation of a nontoxic bacterial pellet from a highly toxic supernatant, the membranes used for the tangential flow filtration do not lead to such a clear-cut separation.

Despite the high molecular weight cut-off, the PSVP molecular filtration membranes were unsuitable for the concentration of *B. pertussis,* because of the complete retention of the toxic factor(s). GSWP microporous membranes gave better results in terms of filtering capacity with respect to this factor(s); its elimination from the concentrate appeared to be facilitated by washings with phosphate buffer.

The results of these experiments indicate that tangential flow filtration using microporous membranes may be very promising for use in the separation of microorganisms from culture broth. The advantages of this system, in comparison with other traditional methods, lie in its low cost, the higher yield and simplicity of use. The filtering unit becomes multi-functional when different types of membranes are adopted, thus allowing additional purification or fractionation steps.

The presence of toxic factor(s) revealed by the concentration of *B. pertussis* for vaccine production purposes, indicates the importance of an appropriate choice of membranes: further studies would appear to be necessary in order to evaluate the possible use of microporous membranes with differing degrees of porosity, and to establish the maximum concentrations that can be reached in consistence with acceptable flow levels, without damaging the integrity and viability of the microorganisms.

Elimination of the residual toxic factor(s) appears possible, however, by a simple centrifugation of the small volumes obtained by tangential filtration of the culture broth.

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Hemopoietic stem cell growth on a capillary stage

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Summary. A hollow fibre capillary stage was used for the maintenance and renewal of hemopoietic stem cells in extra corporeal conditions. The partial success of this technique is due to the preservation of cell-cell contacts and interactions within the tissue sections.

The prolonged survival of hemopoietic stem cells (HSC) in artificial conditions may facilitate studies on their self renewal and terminal differentiation as stimulated by cell-cell interactions and modulated by macrodiffusible differentiating factors.

We describe here a semipermeable cellulose hollow fibre stage which allows mouse spleen HSC extracorporeal survival for at least 12 days. The stage (figure 1) is a support for a monodimensional and coplanar array of 12 fibres into which medium 199 with Hepes (GIBCO) + 10% fetal calf serum was circulated via a multichannel peristaltic pump. On top of the fibres we placed normal DBA/2j mouse spleen sections (10×3.5×1 mm) prepared by slicing spleens held between shimmed glass slides. The sections