| | | | | |
|---|---|---|---|---|
| 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 $1 \times 10^6$ 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

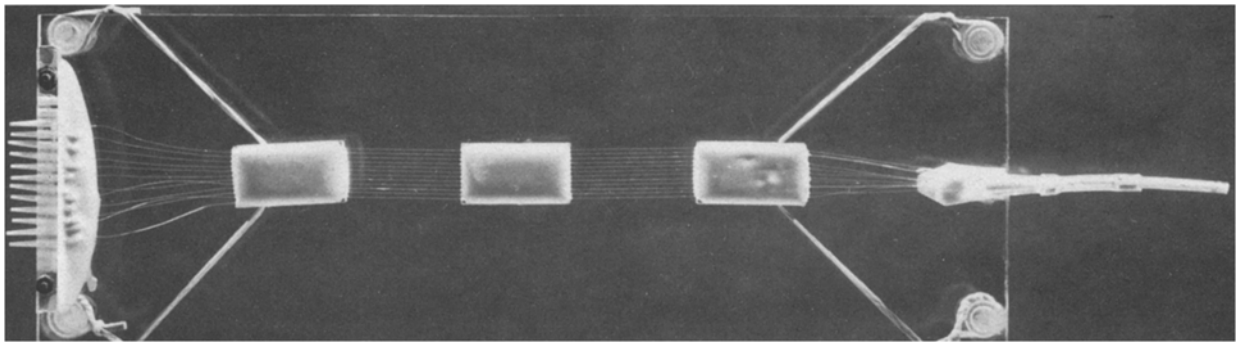

Fig. 1. Top view of the hollow fibre stage (overall dimensions 22 × 7 cm). 12 fibres (inner diameter 180 µm) are strung between supporting posts and spaced 900 µm apart. Each fibre is connected to an individual pump tubing by the inlet coupling (left of picture), into which culture liquid is circulated from a reservoir at the rate of about 0.05 ml/min. The fibres converge into a common outlet whence the liquid returns to the reservoir. A plastic cover, not shown, ensures adequate moisture. In the present configuration 6–8 spleen sections can be positioned crosswise on the fibres between 2 posts. The fibres dialyze isotropically solutes of mol.wt ≤ 5000 daltons. The spleen sections being suspended on top of the fibres are separated from the stage floor by a 5-mm gap. The stage was incubated at 37 °C in moist air with 5% $CO_2$. The medium was changed weekly.

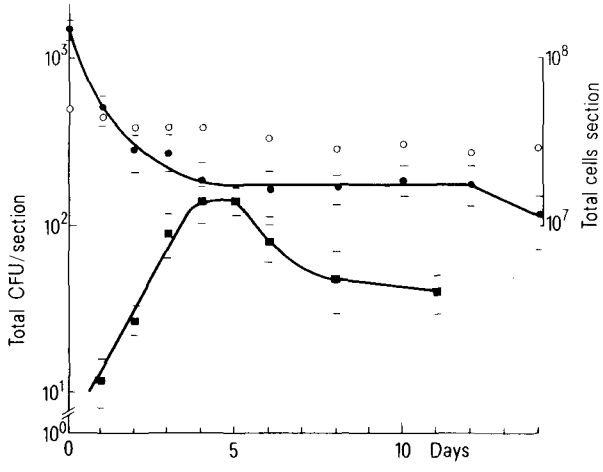


Fig. 2. Survival at various days of CFU within spleen sections cultivated onto the fibre stage. Dots show the CFU maintenance level in sections prepared from normal, non-irradiated adult mouse spleens. This level (178 CFU) is about 12% of the CFU present in the average spleen section at zero time. Open circles show the average total nucleated cells in the same sections. Full squares show the CFU kinetics in spleen sections prepared 1 h after donor mice were whole body irradiated with 250 rad to depress the endogenous spleen CFU content to about 12/section. Stem cell renewal started promptly with a CFU doubling time of 21 ± 5 h. Not shown are the numbers of nucleated cells/section which were quite similar to those reported above. The CFU counts are the average ± SD from 3 experiments for each curve.

were pressed into the fibres to ensure contact with the parenchymal tissue. In this fashion the only foreign surfaces 'seen' by the spleen cells were the fibre walls. This preserved, to some extent, the 3-dimensional structuring of the cells within the sections in contrast to other techniques[2] where tissue sections are plated in culture dishes. This promotes cell migration over the entire dish surface resulting in disassembly of cell complexes and eventual disruption of intercellular cooperation.

The number of HSC surviving at various times within the cultured sections was expressed in terms of colony forming units (CFU) counted as macroscopic spleen colonies on day 8 after cell injection into 950-rad irradiated DBA/2j test mice. Figure 2 shows that after an initial decline from 1500 ± 300 CFU/section the HSC population remained at a level of about 170 CFU/section from day 4 through day 12. We attributed the prolonged, albeit modest, survival of HSC to the preservation, within the sections, of functional coupling between HSC and certain specialized cells, sources of a stimulus for stem cell renewal. The survival of HSC could not have been due solely to the better 'feeding' capacity (e.g. by continuous dialysis) of the fibre stage in terms of macrodiffusible nutrients for, if so, the best feeding regimen should have been obtained by bathing every cell in culture medium. However, when single cell suspensions with 300 CFU were prepared from spleen sections and cultivated in plastic dishes with the same medium used above, the HSC population decreased exponentially, with a half life of 10 h, to undetectable levels (< 5 CFU/section) on and beyond day 3. Such decay is usually observed when HSC are cultivated in suspension using a variety of media and even in vivo in non-hemopoietic tissues deprived of competent sources or microenvironment(s)[3].

To test the ability of the fibre stage in supporting HSC renewal, we sublethally irradiated mice with 250 rad to reduce the CFU content in the spleen sections from 1500 to about 12. We hoped that these endogenous CFU remained functionally coupled to the sources and received optimal stimulation for self renewal. When spleen sections, prepared 1 h after sublethal irradiation, were cultivated on the fibre stage the CFU population increased from 12 to 140 on day 4–5 with a doubling time of about 21 h, similar to the 24 h doubling time for HSC renewing in vivo, and in contrast to other methods, where HSC double only weekly[4]. On subsequent days the CFU content declined to a 'saturation' level of 40–50 CFU/section up to day 11.

The overshoot in CFU numbers (here observed between day 3 and 6) occurs also when HSC are grown in irradiated spleens in vivo[5] and is attributed to a transient overstimulation of the HSC by the irradiated spleen microenvironment[6]. Although on a quantitatively reduced scale, the cultivation of irradiated spleen sections on the fibre stage is thus capable of reenacting the kinetic steps (exponential, overshoot and saturation) experienced by the HSC grown in vivo.

The maintenance and renewal of HSC by the fibre stage should improve if experiments in progress will succeed in increasing the fibre permeability to certain high-molecular-weight factors like erythropoietin, colony stimulating factor, etc. These substances, essential for the development of

macroscopic spleen colonies are now excluded from the sections by the low-molecular-weight cut off ($\leq$ 5000 daltons) of the fibres. In addition, a better stem cell survival shouls be obtained if fouling of the fibres by media with high protein content is reduced.

In spite of these limitations the fibre stage may be employed as an alternative to conventional cell and organ culture systems in a variety of experiments requiring a semipermeable capillary network with known characteristics in terms of structural parameters and dialytic efficiency.

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## Direct measurement of the pH in the stomach of the conscious rat, using a special electrode

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*Summary.* The construction and use of a specially designed electrode to measure gastric pH in the conscious rat is described. Measurement of gastric hydrogen ion activity with this device is rapid and reproducible in starved rats. A dose-related increase of gastric pH was obtained after s.c. injection of the anticholinergic isopropamide.

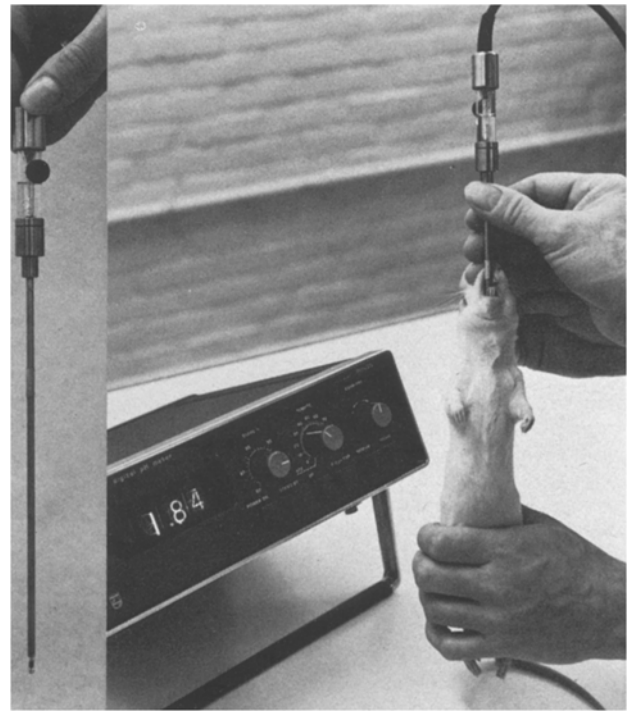
In rats gastric secretion of acid and pepsin remains relatively important in fasting conditions. The measurement of acid secretion in small laboratory animals requires surgical intervention and/or anaesthesia, which by themselves may change the rate and the concentration of acid production. The specially constructed pH electrode presented here allows frequent measurement of the hydrogen ion activity in the stomach of conscious rats.

The pH electrode is a special Philips combined item type CJP built in a stainless steel reinforced glass stem with an outer diameter of 3 mm and a total length of 19.0 cm (figure).

After some training the use of this pH electrode is rapid and easy. 1 operator holds the rat firmly by the skull skin which results in a complete relaxation of the pending animal. A second operator introduces the slender electrode through the throat and oesophagus down so far as to bring a mark on the electrode at the level of the teeth (total depth 13.5 cm in rats weighing $\pm$ 250 g). If some resistance develops, it is usually at the level of the oesophageal sphincter, which can be passed by gentle sidelong movements of the electrode stem. After introduction the pH-meter is switched on and read when the needle reaches a stable position.

A total of 127 male Wistar rats of an inbred strain were used in these experiments and the pH was recorded after a 48-h fasting period. The mean pH $\pm$ SEM in untreated animals was 1.41 $\pm$ 0.015. The distribution of these 127 pH values was normal ($\chi^2 = 2.51$; $q = 4$; $p = 0.64$). All pH readings were between 1.15 and 1.65, with 1 exception of 1.90. To 7 groups of 13 rats each (total 91), the following treatment was applied s.c.: saline (control group) and 0.0025, 0.0050, 0.010, 0.020, 0.040, and 0.080 mg/kg of the anticholinergic isopropamide[2]; 30 min later 1 ml of water (pH 4.5) was given orally by gavage to counteract excessive drying of the mucosal surfaces, and again 30 min later gastric pH was measured. The results of these experiments are summarized in the table. The lowest dose of 0.0025 mg/kg of isopropamide was inactive, whereas 0.005 mg/kg and higher doses induced a significant and dose-dependent increase in pH values. Based on the number of animals reaching a pH $\geq$ 1.75 (which was found in only 1 out of 127 control measurements) the calculated $ED_{50}$[3] was 0.0109 (0.00756–0.0157) mg/kg.

Anticholinergics are able to delay gastric emptying[4], and increase in pH could have been the consequence of a dilution effect of the 1-ml water load retained in the stomach after isopropamide administration. Therefore 7 rats of each treatment group were killed immediately after the pH measurement and their stomachs were removed and weighed. Moreover 7 new groups of 6 rats




pH electrode and its use in the conscious rat.