several α-counting dates, considered in isolation, might. DWBAH shows that the sea rose after 149–129 kyr BP. Harmon *et al.*[2] place cessation of stalagmite growth at 156–112, 171–135 and 182–118 kyr BP. Their coral dates range from 150 to 106 kyr BP; those of ref. 4, 164 to 93 kyr BP ("with no evidence of any discrete, distinct peaks that might be interpreted as specific sea-level maxima"), and ref. 7, 165 to 125 kyr BP. Dates on ooids and peloids[5] range from 169 to 105 kyr BP. These ages cannot be resolved into two populations. There is as yet no evidence for more than one major sea-level rise at stage 5e; under magnification, DWBAH shows continuous deposition between hiatus 2 and hiatus 3.

DWBAH indicates that at ~ 12 m below modern sea level, deposition occurred until ~ 134 kyr BP or later, and did not resume until ~ 110 kyr BP. Any oscillations that may have occurred above −10 m (as shown in ref. 8) would thus not be recorded in DWBAH.

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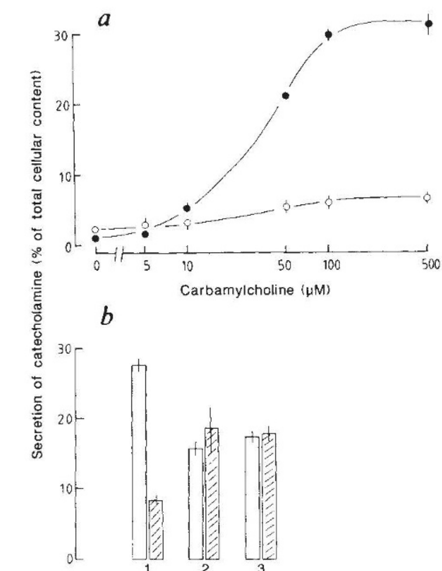
Disposable filters may damage your cells

SIR—Cultured cells are widely and increasingly used in biomedical research. In my own case, I study the mechanism of secretion using primary cultures of bovine adrenal medullary cells. As anyone with experience of tissue cultures will know, there are times when things "don't go right" with cell cultures.

Recently I had one such experience, isolating and plating out chromaffin cells for nearly three months only to find that, within 24 hours of isolation, vacuoles appeared in the cells, many did not attach very well to the plates and those that did responded very poorly to a secretagogue. Their conditions deteriorated steadily over the next few days. After eliminating many possible factors — culture medium, plastics, chemicals used for cell isolation, condition of cattle, and so on — the problem was narrowed down to the disposable syringe filters used for sterilizing the solutions.

The filters were 0.2μ Flowpore Disposable filters D26 series, bought in boxes of 50. Solutions used for washing and disaggregating the cells were sterilized by passage through these disposable filters. Furthermore, culture medium containing various additives were also filtered. When cells were cultured without using these filters, they appeared healthy and secreted catecholamine. Addition to these cells of culture medium passed through the filters halved, within 24 hours, the amount of catecholamine in the culture wells and almost completely abolished the secretory response (*a* in figure).



The effect of filtrate on cultured bovine chromaffin cells. Cells were isolated and cultured as described (Knight, D. E. *FEBS Lett* **207**, 222–226; 1986). After 3 days culture, the medium on the cells was replaced by fresh medium that had or had not been passed once through Flow Pore D26 0.2μ syringe filters. Cells were then washed twice in physiological saline and challenged to secrete with; *a*, various levels of carbamylcholine or *b*, (1) 100 μM carbamylcholine, (2) 150 mM K^+, or (3) a 10 μM Ca^{2+} challenge using CaEGTA buffers, after first rendering the cells leaky for 6 min in potassium glutamate based medium at 0.01 μM Ca^{2+} and containing 10 μM digitonin. The catecholamine secreted over 15 min in response to each of these challenges is shown. *a*, Cells incubated for 18 h in 1 ml per well of filtered culture medium (50 ml culture medium passed once through 5 disposable filters) (○). Control cells incubated for the 18 h in unfiltered culture medium (●). Data are means of 3 determinations ± s.d. *b*, Cells incubated for 3 days in 1 ml per well filtered culture medium (20 ml culture medium passed once through 5 filters (different batch from those in *a*)) (hatched bars). Control cells incubated for the 3 days in unfiltered medium (open bars). Data are means of 6 determinations ± s.d.

The toxicity of the filters was found to vary between batches and sometimes became apparent only after several days of culture. For example, *b* in the Fig. shows the effect of incubating cells for 3 days in the filtrate processed by one such batch of filters. Here the secretory response to carbamylcholine was inhibited by over 50% although the catecholamine content of the cells was unaltered, but secretion was not inhibited when the cells were challenged with high concentration extracellular potassium, which opens voltage dependent calcium channels, or when buffered calcium was introduced directly into the cell after first permeabilizing the membrane with digitonin. These data suggest that the extent of toxicity varies with the stimulus, first affecting receptor mediated responses.

Inquiries by the suppliers, Flow Laboratories Ltd, to the manufacturers Sartorius, revealed that during assembly filters are wetted with polyglycolethers.

Sartorius have now said that they are changing the wetting procedures of the filter assembly in view of the possible toxic effect of polyglycolethers.

Flow Laboratories Ltd are in agreement that the users of these disposable syringe filters should be made aware of possible toxic effects on cell cultures. They suggest that to alleviate the problem with the existing filters, 10 ml of warm distilled water be passed through the filter, followed by a few mls of culture media, before the filter is used for filtration of media destined for cultural purposes.

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NF-κB and HIV

SIR—Griffin *et al.* suggest[1] that the transcription factor NF-κB is activated during differentiation of promonocytic stem cells and that this contributes to the induction of the human immunodeficiency virus (HIV) in latently infected monocytes. Their conclusion is based solely on experimental work with the human promonocytic cell line U937, which is not identical to normal promonocytic stem cells. This approach raises difficulties because transcriptional regulation of individual genes in normal tissue has been shown to differ substantially from regulation in corresponding cell lines[2]. To validate this hypothesis on the basis of observations with U937 cells, it is necessary to study κB binding and transcriptional activity in normal promonocyte and monocyte cells.

Although Griffin *et al.* have correlated HIV gene activation with the appearance of κB-binding activity following induction of U937 cells, previously published experiments[3,4] have demonstrated κB-binding activity in non-induced cells. Both protein binding and *in vitro* transcription assays

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