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R. E. Coupland
A. S. Pyper
D. Hopwood

Department of Anatomy,
University of St. Andrews,
Queen's College, Dundee.

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Toxicity of Cell Culture Medium due to Filtration through Asbestos Pads

During investigations of tissue culture cells under various conditions of storage, it was observed that the viability of a single cell batch varied when tested with different batches of the same medium. This variation was found to be related to the order in which the medium batch was collected during filtration using standard asbestos pads.

The cells used were a hamster fibroblast clone (*C*13) line from the cell line *BHK*21 (ref. 1). They were propagated for 10 days from a frozen cell stock and the comparative tests were all made with the same cell suspension. The growth medium was Eagle's basal medium with two-fold amino-acids and vitamins, plus Difco tryptose broth 10 per cent and uninactivated calf serum 10 per cent. The tryptose broth was autoclaved. Eagle's medium and calf serum were sterilized separately by filtration through standard asbestos compound pads. Each sample of medium was tested in eight 60 mm 'Pyrex' Petri plate cultures, each Petri plate containing 1,000 cells in 5 ml. of medium. Viability was expressed as cloning efficiency, that is, the percentage of cells which formed colonies after 7 days in culture.

Table 1 shows the cloning efficiency of a batch of cells in media made up with a common batch of serum and tryptose broth, but with samples of Eagle's medium taken successively during filtration. It will be seen that the first 500 ml. (or 5 per cent) of the medium filtered led to a marked reduction of cell cloning efficiency, the effect decreasing over the next 1,000 ml. (or 15 per cent) filtered. Thereafter the cell cloning efficiency remained constant.

This effect could be due either to the addition of a toxic factor from the pads, or to the removal of an essential nutrient from the medium by the pad. To distinguish between these possibilities, samples of the first and sixth medium samples were mixed and tested (Table 2). It will be seen that the sixth medium sample was affected by addition of medium from Sample 1 but not by equivalent dilution with 0·9 per cent sodium chloride. This indicates that a toxic factor from the asbestos pad was responsible for the reduction in cell cloning efficiency.

Table 1. Cloning Efficiency of *BHK*21 Cells in Eagle's Medium collected at Successive Intervals during Filtration through Asbestos Pads

Filtrate sample	Sample volume (ml.)	Proportion of total filtrate (%)	No. of colonies (8,000 cells plated)	Cloning efficiency (%)
First	500	5	161	2·0
Second	1,000	10	600	7·5
Third	1,000	10	1,306	16·3
Fourth	2,500	25	1,440	18·0
Fifth	2,500	25	1,425	17·8
Sixth	2,500	25	1,524	19·0

Table 2. Effect of Mixing Early and Late Filtrate Samples of Eagle's Medium on the Cloning Efficiency of *BHK*21 Cells

Filtrate sample	No. of colonies (8,000 cells plated)	Cloning efficiency (%)
First	144	1·8
Sixth	1,547	19·3
Sixth with 10 per cent NaCl 0·9 per cent	1,453	18·2
Sixth with 10 per cent first sample	621	7·8

Similar results were obtained when calf serum was filtered. No change in *p*H was observed in the medium during filtration, and the nature of the toxic factor is unknown.

The phenomenon has obvious practical consequences for cell culture. Asbestos compound pads from three different manufacturers have been tested, and all show toxic activity. Attempts have been made to remove this toxic material by washing with buffered saline and 1 : 20,000 solution of 'Versene'; but these are less efficient than medium itself. It is, therefore, necessary to discard the first part of the filtrate in an amount proportional to the size of the pad, involving, for example, the first 1,000 ml. of fluid from a 14-cm diameter pad.

Unlike asbestos compound pads, cellulose membrane filters (Millipore Filter Corporation, Bedford, Mass.) do not affect the properties of the early filtrates. Ten litres from the batch of Eagle's medium used for the experiment shown in Table 1 were sterilized by filtration through a cellulose membrane instead of asbestos pads. Samples of the filtrate were taken at various stages and tested as previously described. Each sample, including the first 200 ml. of filtrate, gave a cell cloning efficiency of between 18 and 19 per cent.

It is obviously preferable to omit asbestos type filters from all stages of preparation of tissue culture medium and, where possible, to substitute cellulose membrane filters.

W. House

Medical Research Council,
Experimental Virus Research Unit,
Institute of Virology,
University of Glasgow.

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A New Method for Dispersing the Cells of Mammalian Tissues

In 1916 Rous and Jones[1] used trypsin to disperse avian and mammalian cells. Since then some relatively specific proteases, for example, elastase[2] and collagenase[3], have been used, but trypsinization has remained the standard method for this purpose. Trypsin has many drawbacks. Digestion is slow and incomplete, the cells tend to remain in clumps and there is a viscous residue.

Recently, a new protease with unique properties was isolated from the actinomycete, *Streptomyces griseus*. This new enzyme has the broadest substrate specificity of any known protease[4]. Commercially available in a partially purified form under the name 'Pronase' (California Corporation for Biochemical Research, 3625 Medford St., Los Angeles 63, California), it is expected to have a number of applications in the food and drug industries. These applications should soon make it competitive in price with crude trypsin. In basic research, 'Pronase' has already been used for determining the structure of proteins[5] and in experiments with mammalian ova[6,7]. To these applications this communication adds the successful use of 'Pronase' to disperse the cells of mammalian tissues. In our laboratory 'Pronase' has been found to have none of the defects of trypsin. Digestion of both adult and embryonic mouse tissues is rapid and complete, yielding truly monodisperse cell suspensions.

A comparison was made of the relative effectiveness of trypsin (1 : 250, Microbiological Associates) and 'Pronase' for dispersing the cells of 13-day-old Swiss mouse embryos. Both enzymes were prepared as 0·25 per cent (w/v) solutions in phosphate-buffered saline[8], sterilized by 'Millipore' filtration and kept frozen until used. To 50 ml. of each enzyme solution the fragments of 12 embryos were added. Fragmentation of the embryos was achieved by forcing them through a 10 c.c. hypodermic syringe.