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An Autoclavable Powdered Culture Medium for Mammalian Cells (32685)

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Recently, the successful preparation of premixed powdered tissue culture media and their favorable application to virological studies were reported (1,2), and since then some of these media have become commercially available. However, these powdered culture media still require sterilization by filtration before use. The procedure for filter sterilization of these media is time-consuming and laborious, compared with that of the usual bacteriological media. We have prepared a thermostable powdered culture medium that lacks only one component, glutamine. The components of this medium are somewhat modified from that of Eagle's MEM (3), so as to provide a favorable dehydrated state and to permit autoclaving. The present report describes the medium components, the autoclaving procedure, and the cellular growth with this medium.

Table I shows the medium components. Phosphate, calcium, and magnesium salts in the medium were utilized in their anhydrous forms as indicated in the table. The most massive component, sodium chloride, was dehydrated as completely as possible before mixing. Furthermore, choline bitartrate was used instead of the usual choline chloride to avoid the latter's hygroscopic property. The amount of the pH indicator, phenol red, was decreased to 6 mg/liter medium, differing from the original MEM formula, and heat-stable kanamycin was added in the amount of 60 mg/liter medium as an antibiotic before mixing. The special additives in the formulation of the present medium are succinic

TABLE I. Main Components of the Autoclavable Powdered Medium.

Components	mg/liter	gm/100 liters
Arginine	105	10.5
Cystine	24	2.4
Histidine	31	3.1
Isoleucine	52	5.2
Leucine	52	5.2
Lysine	58	5.8
Methionine	15	1.5
Phenylalanine	32	3.2
Threonine	48	4.8
Tryptophan	10	1.0
Tyrosine	36	3.6
Valine	46	4.6
Glucose	1000	100.0
NaCl	6800	680.0
KCl	400	40.0
$CaCl_2$	200	20.0
$MgSO_4$	93.5	9.35
NaH_2PO_4	115	11.5
Kanamycin[a]	*60*	*6.0*
Phenol red	*6*	*0.6*
Sodium succinate	*100*	*10.0*
Succinic acid	*75*	*7.5*
Thiamine	1	0.1
Riboflavin	0.1	0.01
Pyridoxal	1	0.1
Nicotinamide	1	0.1
Pantothenate	1	0.1
Folic acid	1	0.1
Inositol	2	0.2
Choline bitartrate	*1.80*	*0.180*

[a] Italicized components are the ones different from the amounts of original MEM formula or lacking in it.

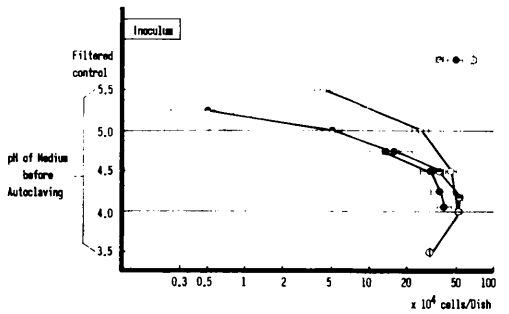

FIG. 1. The comparative yields of cell cultures grown in the autoclaved media at various pH values versus the standard filter-sterilized medium. The open, closed, and half-opened circles represent the mean values of three separate series of experiments where the pH of the medium was adjusted prior to autoclaving.

acid and sodium succinate. As shown in Fig. 1, the medium retains its growth-supporting capacity after being autoclaved as long as it was sterilized at pH 4–4.5. Therefore, succinic acid and succinate were added to the formula as an acidic buffer system. All other components are the same as those of the original MEM prescription. The medium components, except bicarbonate and glutamine (Table I), were premixed with a ball mill, and after being dissolved in aliquot volumes of distilled water were autoclaved just before use. A 10% bicarbonate solution was autoclaved separately in a pressure-plugged vaccine vial, and the 6% glutamine solution was sterilized through a Seitz filter. For the completion of the medium, both sterilized solutions were aseptically added to the above autoclaved main portion of the medium in the amount of MEM formula. The bicarbonate solution should be added to adjust the medium to a pH of 7.2–7.4.

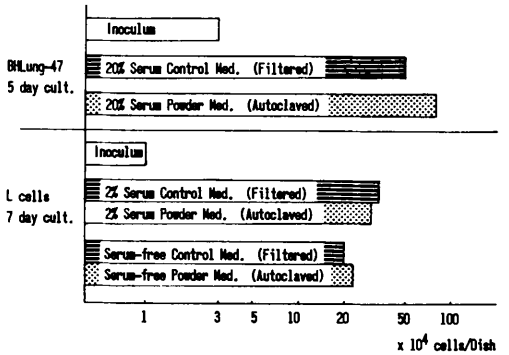

FIG. 2. The comparative yields of baby hamster cells and L cells in both autoclaved and filter-sterilized media. The 5-day cultures of baby hamster lung cells (primary culture) were estimated when grown in a medium containing 20% serum, while the 7-day cultures of L cells were cultivated with 2% serum and seurm-free media.

The growth of baby hamster lung cells (primary culture) and L cells was examined with this medium, and the results are diagrammatically presented in Fig. 2 in comparison with the growth of standard filter-sterilized MEM. As shown in this figure, this autoclavable medium can support the growth of two cell lines in the same manner as filter-sterilized MEM. Moreover, it supported clonal growth of HeLa and L cell lines, with high plating efficiencies, namely, 88–100%. Furthermore, the present medium was observed to support the growth of various human, hamster, and rat cells, normal as well as malignant, and it is now employed as a routine medium in our laboratory.

Summary. An autoclavable powdered tissue culture medium could be successfully prepared by applying its thermostability in acidic pH. The formula of the medium is modified from Eagle MEM so as to be autoclaved at pH 4–4.5. The medium can be used not only for the cloning culture of established cell lines but also for the primary cultures of various mammalian cells.

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