REVERSIBLE ULTRASTRUCTURAL CHANGES IN HUMAN FIBROBLASTS GROWN IN HEPES BUFFERED MCDB-104 SUPPLEMENTED WITH HUMAN SERUM

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SUMMARY

Prolonged maintenance of human dermal fibroblasts in MCDB-104 medium supplemented with pooled human serum or platelet factor deficient preparations of human serum led to appearance of a large number of membrane bound inclusions, resembling lysosomes, and proliferation of small, Golgi associated vesicles. These inclusions did not appear if the cells were grown in Dulbecco-Vogt’s modification of Eagle’s minimal essential medium or in minimal essential medium supplemented with the same human serum fractions. Cells that acquired inclusions during a 10 d incubation in MCDB-104 subsequently lost these inclusions when transferred to Dulbecco-Vogt’s medium for 4 d. Similar reversal of effects of MCDB-104 was also produced by MCDB-104 buffered with bicarbonate instead of HEPES.

Key words: fibroblast; electron microscopy; human serum; growth factors; MCDB-104; HEPES.

INTRODUCTION

Cell culture media commonly in use were developed for culturing specific cell types with specific serum supplements. Eagle’s minimal essential medium (MEM) was developed for culturing HeLa cells in the presence of dialyzed serum (1). Dulbecco-Vogt’s modified Eagle’s medium (DME) was developed for increased growth rates and production of poliomyelitis virus in monkey kidney cells (2). Ham’s F12 medium was developed for clonal growth of Chinese hamster lines in the absence of serum (3), and most recently the MCDB 101-107 series of media have been developed for clonal growth of human fibroblasts in the presence of small amounts of protein from fetal bovine serum (4). Use of these media in culturing cells other than those for which they were designed and with supplements different from those originally proposed is common. The choice of one medium or another for a culture system is often arbitrary and details of the considerations that play a role in this choice usually are not reported.

We have been studying human dermal fibroblasts grown in media containing human serum deficient in platelet derived growth factor (PDGF) or deficient in both PDGF and lipoproteins. Because removal of PDGF and lipoproteins requires several dialysis steps and results in serum depleted of many small molecular weight components, we elected to use MCDB-104 inasmuch as it was developed specifically for culture of human fibroblasts in minimum serum. Morphological characteristics of these cells were monitored using transmission electron microscopy, and our results indicated that dermal fibroblasts developed extensive numbers of inclusions within 10 d in culture in MCDB-104 supplemented with pooled human serum or fractions of pooled human serum (PHS) deficient in PDGF or deficient both in PDGF and lipoproteins. These inclusions did not appear, and ultrastructure appeared normal in all respects if we used MEM or DME supplemented with the same serum or serum fractions. Furthermore, a decrease in the number of these lysosomelike inclusions was ob-
served when the cells were transferred from MCDB-104 to MEM, DME, or MCDB-104 containing bicarbonate buffer instead of HEPES.

**MATERIALS AND METHODS**

Minimal essential medium was prepared from dry powder (GIBCO, Grand Island, NY) following the directions of the manufacturer. Dulbecco-Vogt's modified Eagle's medium was prepared as described previously (5) in the Department of Pathology at the University of Washington. It was formulated as described (2,5) except that it contained 4,500 mg/l glucose and 2,250 mg/l NaHCO₃. MCDB-104 (4) was purchased from GIBCO. One batch that had precipitated before delivery was returned; all others were free of precipitation. MCDB-104 with bicarbonate was purchased from GIBCO and differed from MCDB-104 in the following components: no HEPES, no sodium hydroxide, 1,176 mg/l sodium bicarbonate, 7,599 mg/l sodium chloride. All media were supplemented with 100 μg/ml neomycin (GIBCO).

Serum and serum fractions were prepared from pools of blood from three to six normal males aged 25 to 40. After informed consent was obtained, blood was collected by venipuncture and processing was begun with 1 h. Use of stored blood or blood with specific blood group antigens was not evaluated. Pooled human serum was prepared from blood allowed to clot for 2 h at 37°C; after centrifugation at 1000 ×g for 20 min at 4°C it was frozen and not treated further. Platelet derived growth factor deficient serum (PDS) was prepared as described previously (6). Plasma was obtained from blood anticoagulated with citrate-phosphate-dextrose USP by two centrifugations: 1,000 ×g for 20 min, and 22,000 ×g for 30 min. It was subsequently recalcified (20 mM CaCl₂) allowed to clot at 37°C for 2 h, and centrifuged at 22,000 ×g for 20 min to remove fibrin. After dialysis against 0.1 M Tris, pH 7.4, it was chromatographed on CM Sephadex C-50 (Pharmacia, Piscataway, NJ) to remove residual basic proteins including platelet mitogen, concentrated by ultrafiltration (Amicon, Lexington, MA).

**FIG. 1.** Electron micrograph of a fibroblast grown for 10 d in MEM supplemented with PHS. Cells contain dilated RER, numerous pinocytotic vesicles (arrows), and a small number of membrane bound inclusions containing heterogeneous electron dense material (L). These cells resemble those grown in MEM or DME supplemented with PDS, MEM, or DME supplemented with PDLDS. ×9,000.