Long-Term Cultivation of Human Cells (Chang) in Chemically Defined Medium and Effect of Added Peptone Fractions. By Richard Holmes. (*From the Alfred I. du Pont Institute of The Nemours Foundation, Wilmington, Delaware.*)*

Freshly explanted tissue cells and most established cell lines cannot be cultivated *in vitro* indefinitely in chemically defined medium unsupplemented with serum (1). Yeast extract and peptone preparations are sources of growth stimulatory material which can alternate for serum in a limited number of cases (1, 2). To identify unequivocally the stimulatory components of either serum or peptone requires that the test system include no other unknown material. Thus, there is need for a chemically defined medium that will support continuous growth of cells, but whose growth potential can be increased by adding the serum or peptone components.

TABLE I

Composition of Medium A

Composed of Medium 858 devised by Healy, Fisher, and Parker (*Proc. Soc. Exp. Biol. and Med.*, 1955, **89,** 71), modified as follows:

Cysteine	75.0	According to:
Glutathione	15.0	Waymouth
Ascorbic acid	17.5	*J. Nat. Cancer Inst.*, 1956, **17,** 315.
Pyrodoxine	0.025	
Thiamine	0.010	Eagle
Riboflavin	0.010	*Proc. Soc. Exp. Biol. and Med.*, 1956, **91,** 358.
Ca pantothenate	0.010	*Science*, 1956, **123,** 845.
Inositol	10.0	
Fructose 1-6 phosphate	100.0	Fischer *et al.* *Proc. Soc. Exp. Biol. and Med.*, 1948, **67,** 40.
Hypoxanthine	1.0	
Xanthine	1.0	
Adenosinetriphosphate	1.040	Morgan *et al.*
Adenosine 5′-phosphate	0.020	*J. Nat. Cancer Inst.*, 1956, **16,** 1405.
D-Ribose	0.2	
D-Deoxyribose	0.2	
1-Alpha amino butyric	2.500	
1-Asparagine	4.100	
1-Ornithine	3.500	Evans *et al.*
1-Taurine	2.000	*Cancer Research*, 1956, **16,** 77.
D-Glucosamine	3.200	
Glucuronolactone	1.000	
1-Homocystine	1.0	
Ergothionine HCl	1.0	
Glucose 1-phosphate	1.0	
Citric acid	1.0	Other components
Beta-estradiol	0.100	
Thioctic acid	1.500	
Cholic acid	20.0	
Carboxymethyl cellulose 70L	250.0	

Whenever practical, purity of ingredients has been checked by paper chromatography and by electrophoresis.

Medium A, shown in Table I, has been found to support continuous growth (11 months to date) of

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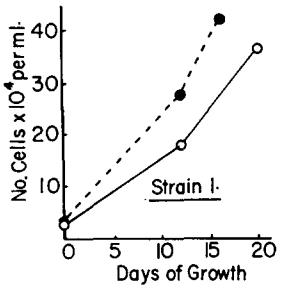

FIG. 1. Growth of strain 1 adapted human liver epithelial cells in Medium A (solid line), and in Medium A supplemented with 0.1 per cent peptone (broken line). Each culture was started with 30 × 10^3 cells per ml. that had been washed in Medium A.

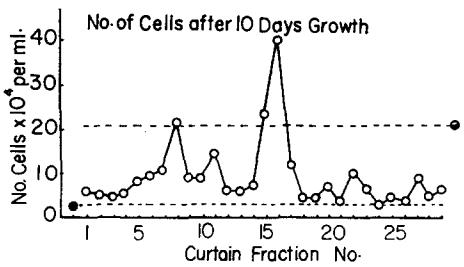

FIG. 2. Effect of electrophoretically prepared peptone fractions on the growth of cells. Each culture was started with a washed replicate inoculum of cells and a different peptone fraction (0.1 per cent) dissolved in Medium A. The number of cells found after 10 days of growth in Medium A alone is represented by lower dotted line, and in Medium A plus 0.1 per cent whole peptone by upper dotted line. Peptone fraction 1 is acidic; fraction 29 is basic. The material in fractions 15 and 16 is heat stable, dialysable, and could be separated by paper chromatography into three major components that reacted with ninhydrin.

Chang's human liver epithelial cells. Two strains of Chang cells were obtained that grew in Medium A after 120 days of adaptation, but the first strain did not grow as rapidly as the second. The course of adaptation was similar to that originally described for the L strain mouse fibroblast (3). Chang cells (Microbiological Associates) grown in Medium A supplemented with 20 per cent by volume, adult bovine serum, were rinsed twice with Medium A, removed from the glass surface of the original T-30 flask by means of a scraper, and divided equally between two T-30 flasks. When a confluent sheet of cells grew out on the surface of the new flasks the cells were again transferred to two flasks. One-half the total volume of medium in each flask was replaced with fresh Medium A three times a week; free floating cells and debris were removed along with the spent medium. Growth was rapid for two to three transfers, after which the described adaptation period occurred before growth resumed. The addition of 0.1 grams of bacto peptone (Difco) per 100 ml. of Medium A reduced the adaptation period to 90 days. Fig. 1 compares the rate of growth of strain one of adapted Chang cells in Medium A alone and in Medium A plus 0.1 per cent peptone, commencing with 30 × 10^3 cells per ml. The stimulatory action of peptone was not reduced by autoclaving.

Chang cells cultivated in Medium A were used to test the stimulating effect of peptone fractions prepared by curtain electrophoresis (4) with sodium bicarbonate electrolyte (5) 0.0125 M, pH 8.4. To each of a series of T-30 culture flasks was added 4 ml. of Medium A containing 15 × 10^3 cells per ml. Each flask was further supplemented with 0.04 grams of one of the peptone curtain fractions. (These fractions were prepared by lyophilization of the respective curtain fractions after neutralization of sodium bicarbonate with an equivalent amount of HCl). All flasks were incubated at 37°C. and half the volume of medium in each flask was replaced 3 times a week. At the end of 10 days the cells in four different low power microscope fields of each flask were counted. The number of cells per ml. was plotted against the respective curtain fraction numbers. One set of results representative of three separate experiments is shown in Fig. 2. These results, which not only compared the ability of peptone substances of different electrophoretic mobility to stimulate the growth of mammalian cells, also illustrated a method for their partial purification.

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