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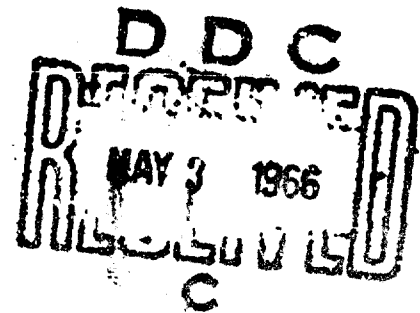
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TECHNICAL MANUSCRIPT 282**DIFFERENCES IN PROTEIN CONTENTS
AMONG FIBROBLAST CELL LINES
GROWN IN MONOLAYER CULTURES**

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**UNITED STATES ARMY
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DIFFERENCES IN PROTEIN CONTENTS AMONG FIBROBLAST CELL LINES
GROWN IN MONOLAYER CULTURES

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ABSTRACT

The changes in cell protein values of cat kidney and McCoy's human synovial cell lines were determined during various phases of monolayer growth in a lactalbumin hydrolyzate - 10% calf serum medium. The McCoy's cell protein values ranged from 230×10^{-6} to 574×10^{-6} $\mu\text{g}/\text{cell}$, whereas the cat kidney cell values ranged from 131×10^{-6} to 367×10^{-6} $\mu\text{g}/\text{cell}$. Maximum values for each cell line were recorded during a short period in the lag phase of growth. Essentially constant minimum values were observed after the cultures reached a slowly proliferating, densely populated, post-confluent state. For this reason, the characteristic protein values reported for each cell line were determined during the latter part of the logarithmic growth phase.

An effect of composition of growth medium on cell protein values was observed. When cultured in a serum-free chemically defined medium, the protein values during the latter part of the logarithmic growth period averaged $281 (\pm 28) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for McCoy's cells, $171 (\pm 18) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for L cells, and $134 (\pm 18) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for cat kidney cells. On the other hand, when grown in a lactalbumin hydrolyzate - 10% calf serum medium, the protein values averaged $249 (\pm 20) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for McCoy's cells, $278 (\pm 35) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for L cells, and $139 (\pm 33) \times 10^{-6}$ $\mu\text{g}/\text{cell}$ for cat kidney cells.

It is believed that these measurements may serve, under suitable standardized conditions, as a method for the characterization of cell lines.

I. INTRODUCTION

Studies on the protein contents of mammalian cells cultured in vitro have been described by Oyama and Eagle,¹ by Salzman,² by Swaffield and Foley,³ and by Miedema and Kruse.⁴ The present report is an extension of their work with a view toward application of such data to characterization of cell lines.*

II. MATERIALS AND METHODS

A. CELL LINES

Four fibroblast cell lines designated as being derived from cat kidney (CK), human synovial tissue (MC for McCoy), hamster kidney (HK), and mouse (L) were used in these experiments. These cells have been maintained in serial transfers for several years in Medical Bacteriology Division. Cultures were propagated for a number of passages in either a chemically defined medium or in a lactalbumin hydrolyzate - 10% calf serum medium before their use as inocula in the respective growth medium.

B. MEDIA AND CULTURE METHODS

The compositions of the serum-free chemically defined medium and the lactalbumin hydrolyzate - 10% calf serum medium are presented in Tables 1 and 2, respectively. These are modifications of media described previously.** Media were prepared by standard procedures. Concentrated stock solutions of vitamins, antibiotics, carbohydrate, amino acids, and salts were combined and sterilized by filtration through membrane*** filters, 0.22 μ pore size.

* Galacci, R.R.; and Galacci, R.R.; Higuchi, K. March, June, Sept. 1965. MB Division Quarterly Technical Status Reports, Nutrition Branch, Tissue Culture Section.

** Higuchi, K. December 1958. MB Division Quarterly Technical Report, Nutrition Branch.
Higuchi, K. March 1964. MB Division Triannual Report, Nutrition Branch, Tissue Culture Section.

*** Millipore Filter Corp., Bedford, Mass.

TABLE 1. SERUM-FREE CHEMICALLY DEFINED MEDIUM FOR GROWTH OF FIBROBLAST CELL LINES IN MONOLAYER CULTURE

Component	Concentration, mg/liter	Component	Concentration, mg/liter
L-Amino Acids		Salts	
Arginine·HCl	105	NaCl	7400
Asparagine	193	KCl	400
Cysteine·HCl	78	NaH ₂ PO ₄ ·H ₂ O	100
Glutamine	438	NaHCO ₃	1140 ^{b/}
Histidine·HCl	58	CaCl ₂ ·2H ₂ O	265
Isoleucine	131	MgCl ₂ ·6H ₂ O	275
Leucine	262	Fe(NH ₄)(SO ₄) ₂ ·12H ₂ O	0.97
Lysine·HCl	274		
Methionine	60	Carbon sources	
Phenylalanine	116	Glucose	1800
Proline	200	Sodium pyruvate	110
Serine	158	Gluconolactone	178
Threonine	119		
Tryptophane	61	Vitamins	
Tyrosine	118	D-Biotin	2.5
Valine	147	C. Choline Cl	2.5
Antibiotics and Misc.		Folic acid	2.5
Streptomycin	100	Niacinamide	2.5
Penicillin	63	Thiamine·HCl	2.5
Kanamycin	100	Ca-pantothenate	5.0
Phenol red	10	L-Inositol	2.5
Methocel, 15 cps	500	Pyridoxal·HCl	2.5
Hemin	0.30	B ₁₂	0.005
N.P.H. Insulin	(5 U/l) ^{a/}	Riboflavin	0.25

a. Units per liter.

b. Of this amount, 840 mg were added aseptically after sterilization (by filtration) and dilution of medium.

TABLE 2. LACTALBUMIN HYDROLYZATE - 10% CALF SERUM MEDIUM FOR GROWTH OF FIBROBLAST CELLS IN MONOLAYER TISSUE CULTURE

Component ^{a/}	Concentration, mg/liter	Component	Concentration, mg/liter
Carbon and nitrogen sources		Salts	
Lactalbumin hydrolyzate	2500	NaCl	7400
Glucose	2000	KCl	400
Sodium pyruvate	110	NaH ₂ PO ₄ ·H ₂ O	100
L-Glutamine	300	NaHCO ₃	972 ^{b/}
		CaCl ₂ ·2H ₂ O	265
		MgCl ₂ ·6H ₂ O	275
Antibiotics and Misc.		Vitamins	
Streptomycin	100	D-Biotin	2.5
Penicillin	63	Choline Cl	2.5
Kanamycin	100	Folic acid	2.5
Phenol red	10	Niacinamide	2.5
		Thiamine·HCl	2.5
		Ca-pantothenate	5.0
		l-Inositol	2.5
		Pyridoxal·HCl	2.5
		B ₁₂	0.005
		Riboflavin	0.25

a. The medium was fortified with 10% calf serum.

b. Of this amount, 672 mg were added aseptically after sterilization (by filtration) and dilution of medium.

The cells were inoculated into replicate 25 cm² Falcon tissue culture flasks, each containing 5 ml of medium, and incubated under static conditions at 36 C. Fresh medium was introduced daily. Cells were harvested at the intervals indicated by dispersion of monolayers with a combination of trypsin (0.01%) and EDTA (0.01 M). Duplicate 2-ml aliquots of cell suspension from each flask were centrifuged at 3,000 rpm for one minute. One sample of cells was resuspended in 2 ml of fresh growth medium to obtain the cell count in a hemocytometer. The cells in the duplicate centrifuge tube were washed and resuspended in 2 ml of the alkaline copper reagent of Oyama and Eagle¹ and stored at 5 C until protein determinations were made. Zero time protein assays of the inoculum were also made. The protein value per cell for each culture was calculated by dividing the cell protein value for the culture in µg/ml by the cell count/ml. The protein values and cell counts were then plotted and compared.

III. RESULTS

A. CHANGES IN PROTEIN CONTENT DURING THE GROWTH CYCLE

At inoculation, each flask for the experiment contained 0.30×10^6 MC cells per square centimeter of growth surface (Fig. 1). The cell protein content at zero time was 332×10^{-6} μ g/cell. After one day, while in the lag phase, a maximum value of 574×10^{-6} μ g/cell was obtained. The protein values then decreased to a constant minimum value by the fifth day.

A similar fluctuation in the protein contents of the CK fibroblast cell line is illustrated in Figure 2. At inoculation each flask contained 0.35×10^6 CK cells/cm². The protein content per cell at zero time was 179×10^{-6} μ g/cell. After 2 days a maximum value of 367×10^{-6} μ g/cell was obtained; then the protein value gradually decreased to a constant value by the seventh day. Also demonstrated in Figure 2 is the difference between protein values obtained by our technique and the technique of Oyama and Eagle.¹ By the latter technique in which the cells are not trypsinized, higher protein values were consistently obtained.

B. DIFFERENCES IN PROTEIN VALUES AMONG CELL LINES

The protein contents of four cell lines obtained during the latter part of the logarithmic growth in the chemically defined medium are presented in Table 3. The protein values averaged $281 (\pm 28) \times 10^{-6}$ μ g/cell for MC cells, $219 (\pm 17) \times 10^{-6}$ μ g/cell for HK cells, $171 (\pm 18) \times 10^{-6}$ μ g/cell for L cells, and $134 (\pm 18) \times 10^{-6}$ μ g/cell for CK cells. Additional data presented in Table 4 show cell line differences in protein values resulting when cells were grown in the lactalbumin hydrolyzate - 10% calf serum medium.

C. EFFECT OF GROWTH MEDIUM

A comparison of data presented in Tables 3 and 4 revealed the effects of growth medium on the protein contents of L cells, CK cells, and MC cells. A significant difference in cell protein value was noted between the two media in the case of the L cells. Protein values for L cells averaged $278 (\pm 27) \times 10^{-6}$ μ g/cell when grown in the lactalbumin hydrolyzate - 10% calf serum medium, and $171 (\pm 18) \times 10^{-6}$ μ g/cell when grown in the chemically defined medium. No appreciable difference in cell protein occurred in CK cells grown in the two media. The CK cell values averaged $139 (\pm 30) \times 10^{-6}$ μ g/cell for cells grown in the serum medium and $134 (\pm 18) \times 10^{-6}$ μ g/cell for cells grown in the defined medium. MC cells, in contrast to L cells, yielded higher protein values in the defined medium. The MC values averaged $249 (\pm 20) \times 10^{-6}$ μ g/cell for cells grown in serum medium, and $281 (\pm 28) \times 10^{-6}$ μ g/cell for cells in the defined medium.

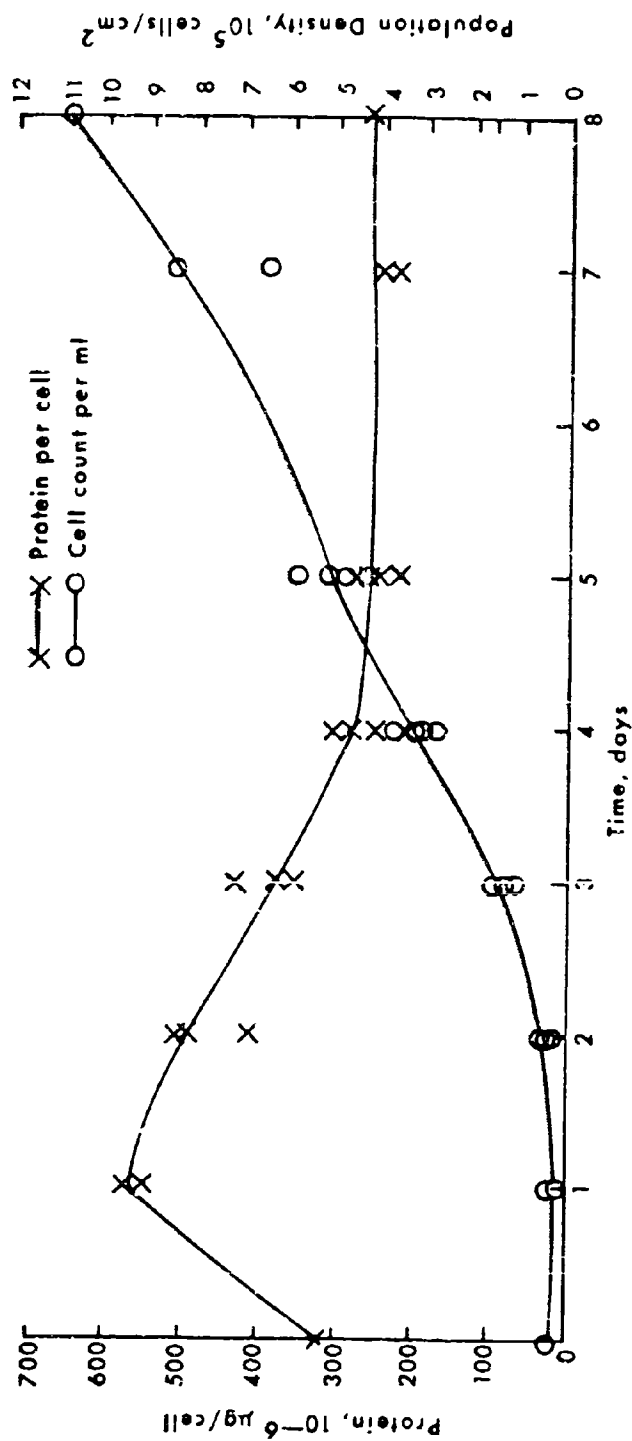


Figure 1. Changes in Protein Content During the Growth Phases of McCoy's human synovial cells in a Lactalbumin Calf Serum Medium.

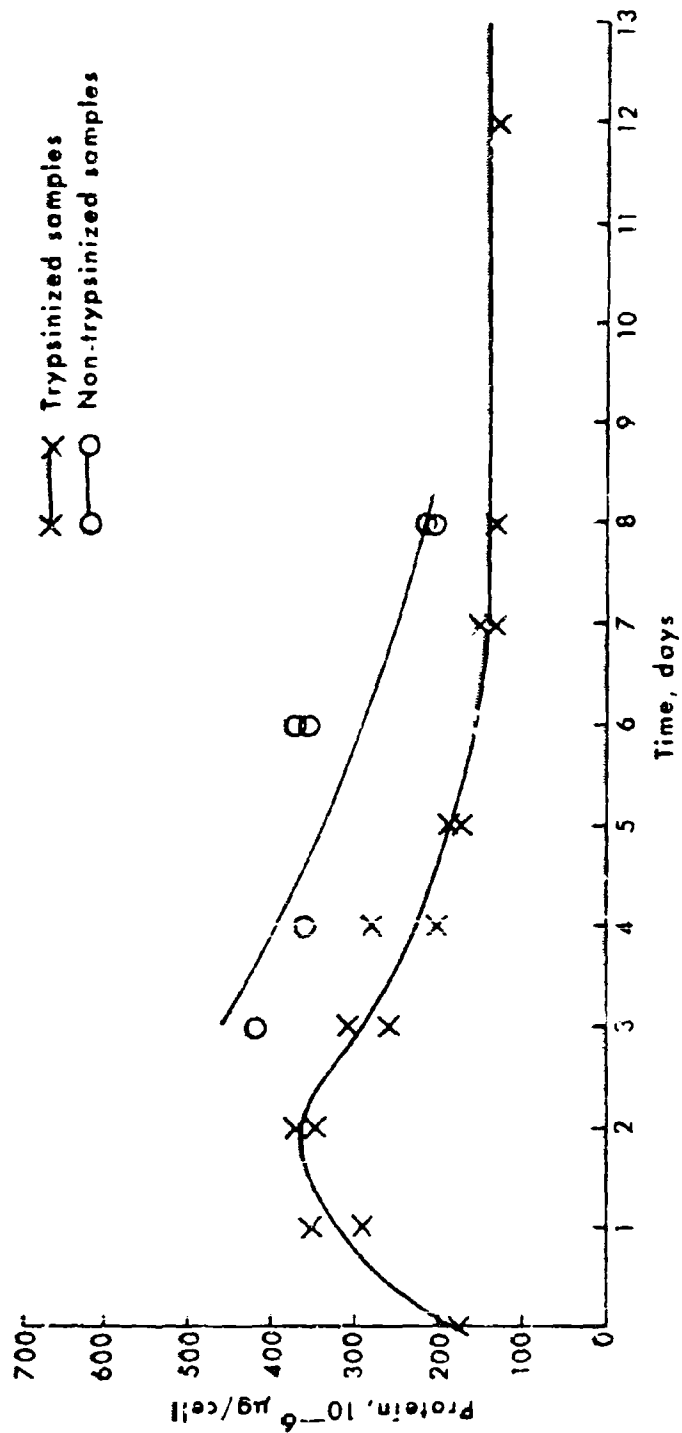


Figure 2. Changes in Protein Content During the Growth Phases of Cat Kidney Cells in a Lactalbumin Galf Serum Medium.

TABLE 3. CELL PROTEIN CONTENTS OF FIBROBLAST CELL LINES GROWN IN THE CHEMICALLY DEFINED MEDIUM

Cell Line ^a /	Maximum Population, $\frac{b}{10^5}$ cells/cm ²	Protein, $\frac{c}{10^{-6}}$ μ g/cell	Average Protein, 10^{-6} μ g/cell
Human Synovial (MC)	3	309, 273, 259, 288, 253, 303	281 (± 28)
Hamster Kidney (HK)	2.2	202, 215, 231, 228	219 (± 17)
Mouse (L)	15.4	167, 153, 162, 176, 171, 181, 154, 187, 188	171 (± 18)
Cat Kidney (CK)	13.8	125, 138, 152, 134, 131, 139, 122	134 (± 18)

- a. Data from three separate experiments are presented for each cell line except HK. Only one experiment was run for HK.
- b. The highest population densities reached during the determination of cell protein values.
- c. The protein values were determined during the latter part of the logarithmic growth phase of the cultures.

TABLE 4. CELL PROTEIN CONTENTS OF FIBROBLAST CELL LINES GROWN IN THE LACTALBUMIN HYDROLYZATE - 10% CALF SERUM MEDIUM

Cell Line ^a	Maximum Population, ^b / 10 ⁵ cells/cm ²	Protein, ^c / 10 ⁶ µg/cell	Average Protein, 10 ⁶ µg/cell
Mouse (L)	7.7	250, 254, 288, 297, 303, 276	278 (±27)
Human Synovial (MC)	8.1	256, 245, 249, 230, 269	249 (±20)
Cat Kidney (CK)	14.0	128, 159, 155, 131, 133	139 (±30)

a. Data from two separate experiments are presented for each cell line.

b. The highest population densities reached during the determinations of cell protein contents.

c. The protein values were determined during the latter part of the logarithmic growth phase of the cultures.

D. MAXIMUM POPULATION DENSITIES OF CELL CULTURES

The protein values reported in Tables 3 and 4 were determined when the cultures were in a slowly proliferating, densely populated, post-confluent state. The maximum population densities reached by the cells grown in the chemically defined medium were 3×10^5 cells/cm² for MC cells, 2.2×10^5 cells/cm² for HK cells, 15.4×10^5 cells/cm² for L cells, and 13.8×10^5 cells/cm² for CK cells (Table 3). The maximum population densities reached by cells grown in the lactalbumin hydrolyzate - 10% calf serum medium were 7.7×10^5 cells/cm² for L cells, 8.1×10^6 cells/cm² for MC cells, and 14×10^5 cells/cm² for CK cells (Table 4).

IV. DISCUSSION

We employed a modification of the method of Oyama and Eagle¹ for the determination of the protein contents of mammalian cells cultured in vitro. Swaffield and Foley³ using a different modification of the procedures of Oyama and Eagle, and Salzmann² employing a UV spectrophotometric method also described systematic fluctuations in protein contents of mammalian cell cultures during growth. More recently Miedema and Kruse⁴ using the method described by Oyama and Eagle¹ have reported similar changes in protein values obtained with fibroblast cell lines. The protein content of cultured cells reported by all of these authors are higher than those obtained in the present work. However, it must be pointed out that in their work, different cell lines were employed; and moreover, our analytical techniques were also different. In our procedure the cells were trypsinized prior to assay for protein.

Because of the possibility that the variances described above might be attributed to a discrepancy in the protein standard used by us, comparisons were made of our protein standard (crystalline bovine serum albumin) with a commercially available protein standard (Pro-Scl),* and also with crystalline egg albumin, but no significant differences were observed. These comparisons provided assurance that our analytical standards were reliable.

The data reported in Table 3 and Table 4 show the importance of using a standard medium for the determinations of protein content per cell. The results generally varied greatly with the culture medium employed; therefore, it is recommended that a chemically defined medium be used whenever feasible in cell protein studies.

* Standard Scientific Supply Corporation, New York, N.Y.

Because of the limited number of cell lines examined, and the clear differences in protein values among them, it was possible to identify each of the four cell lines from each other on the basis of their cell protein values. As more cell lines are examined, however, there will be overlapping of values. Nevertheless it is believed that this method can be of considerable value in many laboratories that require a method for identifying one cell type among several others of similar morphological appearance.

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