

UNCLASSIFIED

AD NUMBER
AD866111
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; FEB 1970. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.
AUTHORITY
BDRL D/A ltr, 29 Sep 1971

THIS PAGE IS UNCLASSIFIED

AD

AD 866111

TECHNICAL MANUSCRIPT 591

REQUIREMENTS FOR CHOLESTEROL,
HEMATIN, AND LECITHIN
FOR OPTIMAL GROWTH
OF A PORCINE KIDNEY CELL LINE

Kiyoshi Higuchi

FEBRUARY 1970

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/TID. Frederick, Maryland 21701

DEPARTMENT OF THE ARMY

Fort Detrick
Frederick, Maryland

D D C
RECORDED
MAR 16 1970
REGULATED
C

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information Springfield Va. 22151

49802

ACCESSION NO.		
OPS//	WRITE SECTION	<input type="checkbox"/>
DDC	BOFF SECTION	<input checked="" type="checkbox"/>
UNANNOUNCED		<input type="checkbox"/>
JUSTIFICATION		
BY		
DISTRIBUTION/AVAILABILITY CODES		
DIST.	AVAIL. STG. OR SPECIAL	
2		

Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Release Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 591

REQUIREMENTS FOR CHOLESTEROL, HEMATIN, AND LECITHIN
FOR OPTIMAL GROWTH OF A PORCINE KIDNEY CELL LINE

Kiyoshi Higuchi

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602AD01

February 1970

REQUIREMENTS FOR CHOLESTEROL, HEMATIN, AND LECITHIN FOR OPTIMAL GROWTH
OF A PORCINE KIDNEY CELL LINE*

ABSTRACT

The nutritional requirements for growth of a porcine kidney (PK) cell line in a chemically defined medium were studied in cells grown as monolayer cultures in T-30 Falcon plastic flasks incubated at 36 C with caps tightened. The PK cell appeared to be unique among a variety of heteroploid cell lines in its requirement for a number of unusual substances. Successful propagation of PK cells was obtained in a serum-free defined medium that contained cholesterol (2×10^{-5} M), hematin (0.5 $\mu\text{g/ml}$), lecithin (2 $\mu\text{g/ml}$), and coenzyme Q_{10} (1 $\mu\text{g/ml}$). The PK cell line may serve as a useful tool in a study of intermediary lipid metabolism at the cellular level.

Bailey¹ showed that several established mammalian cell lines were capable during growth of synthesizing cellular lipids from simple precursors such as glucose and acetate. Radioactively labeled precursor carbon was recovered in cholesterol, triglycerides, and phospholipids. Bailey also showed that these cells were able to utilize lipids supplied exogenously when placed in a growth medium containing serum. On the other hand, Sato and co-workers² reported that cholesterol was required at a concentration of 1 $\mu\text{g/ml}$ for efficient cloning of HeLa S3 cells in a medium containing highly dialyzed serum. Lockart and Eagle,³ however, found that cholesterol did not enhance the growth of isolated single HeLa cells in a similar experiment. They attributed the contradictory results to differences in methods of inoculum preparation. An alternative explanation that can be considered is that differences in degree of dialysis of serum caused failure to demonstrate a need for cholesterol. Cloning presents exacting conditions with respect to nutritional requirements; it is therefore not surprising that a requirement for cholesterol by continuous cell lines had been detected previously only in cloning experiments.

The above results indicate that a requirement for cholesterol could be demonstrated only under special conditions. Recently, however, Holmes and co-workers⁴ reported that human diploid cell cultures grew only if cholesterol was supplied at levels of 1 mg/liter in a defined medium fortified with purified serum fractions. These workers suggested that a requirement for cholesterol was characteristic of primary diploid mammalian cells and implied that heteroploid cells do not require the substance in their growth medium.

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

Work in our laboratory has been devoted to studies on the cultivation of established cell lines in various chemically defined media. A variety of cell lines has been grown successfully in a rather simple medium whose composition is shown in Table 1. Our results, therefore, were in accord with the hypothesis of Holmes and co-workers. During the past year, however, a porcine kidney (PK) line described by Inoue and Ogura⁵ failed to grow in our basal defined medium. A variety of substances were tested in order to promote growth of PK cells; among these, cholesterol, lecithin, hematin, and coenzyme Q₁₀ appeared to improve growth significantly. A combination of these substances when added to the basal medium permitted indefinite serial passages of PK cells, even with inoculum as low as 20,000 cells/ml.

TABLE 1. COMPOSITION OF THE CHEMICALLY DEFINED MEDIUM

<u>L amino acids</u>	mg/liter	<u>Vitamins</u>	mg/liter
arginine·HCl	32	D-biotin	1.0
asparagine·H ₂ O	150	choline Cl	1.0
cysteine HCl·H ₂ O	22	D-Ca-pantothenate	2.0
glutamine	196	folic acid	1.0
histidine·HCl·H ₂ O	63	myo-inositol	1.0
isoleucine	33	pyridoxal HCl	1.0
leucine	26	riboflavin	0.1
lysine·HCl	28	thiamin HCl	1.0
methionine	15	vitamin B ₁₂	0.002
phenylalanine	33	nicotinamide	1.0
proline	115		
serine	105	<u>Salts</u>	
threonine	12	NaCl	7400
tryptophan	6.3	KCl	400
tyrosine	46	NaH ₂ PO ₄ ·H ₂ O	100
valine	35	NaHCO ₃	670
		CaCl ₂ ·2H ₂ O	148
<u>Misc. substances</u>		MgCl ₂ ·6H ₂ O	305
glucose	1800	Na-pyruvate	110
gluconic acid	178	FeNH ₄ (SO ₄) ₂ ·12H ₂ O	4.85
methylcellulose (15 cps grade)	500	ZnSO ₄ ·7H ₂ O	0.288
phenol red	10		
insulin (lente Iletin)	(0.05 U/ml)		
penicillin G-sodium	67		
streptomycin SO ₄	100		

The growth response of PK cells to graded levels of cholesterol in a medium containing coenzyme Q_{10} but no hematin or lecithin is shown by data plotted in Figure 1. Cultures were inoculated with 62,000 cells per ml in Falcon T-30 plastic flasks and incubated at 36 C for 1 week. Media were replaced twice during the growth cycle. Cell protein values were determined by the method of Oyama and Eagle⁶ with crystalline bovine serum albumin as the protein standard. Approximately 1×10^{-5} M cholesterol (corresponding to about 4 $\mu\text{g}/\text{ml}$) produced peak yields of 114 μg of cellular protein per ml of growth medium.

Of the four substances mentioned above that were tested, cholesterol yielded the greatest enhancement of growth of PK cells. The results of single additions of hematin, lecithin, and coenzyme Q_{10} to the basal defined medium containing 2×10^{-5} M cholesterol are presented in Table 2. Both hematin (0.5 $\mu\text{g}/\text{ml}$) and lecithin (2 $\mu\text{g}/\text{ml}$) produced significant improvement in growth of PK cells in a medium containing cholesterol. Coenzyme Q_{10} appeared to have no stimulatory effect in this test; however, in other experiments, this compound seemed to benefit the growth of PK cells.

TABLE 2. EFFECTS OF HEMATIN, LECITHIN, AND COENZYME Q_{10} ON GROWTH OF PORCINE KIDNEY CELL LINE

Test Substance Added to Basal Medium ^{a/}	Cell Protein Yield, ^{b/} $\mu\text{g}/\text{ml}$
None	127, 114 (120)
Hematin (0.5 $\mu\text{g}/\text{ml}$)	232, 265 (298)
Lecithin (2 $\mu\text{g}/\text{ml}$)	242, 237 (240)
Coenzyme Q_{10} (1 $\mu\text{g}/\text{ml}$)	80, 104 (97)
All three (as above)	307, 343 (327)

a. The basal medium contained cholesterol at 2×10^{-5} M.

b. Averages in parentheses.

To our knowledge, the stimulatory effects of hematin and lecithin on growth of cultured animal cells have not been reported previously. Further studies will be made to determine more precisely the concentrations of these substances needed for optimal growth of PK cells.

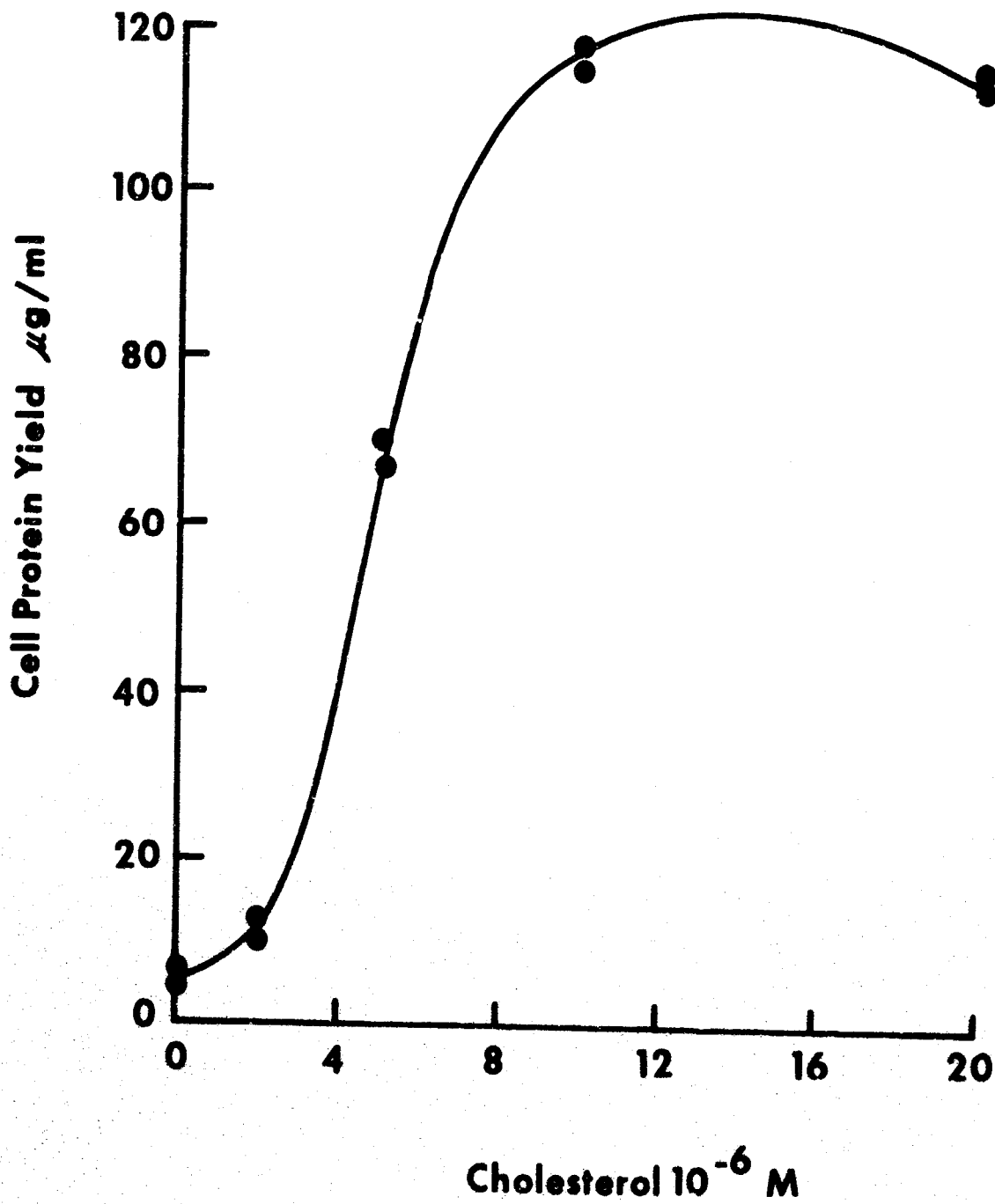


FIGURE 1. Growth Response of Porcine Kidney Cell Line to Cholesterol.

In summary, we have obtained excellent growth of the porcine kidney (PK) cell line described by Inoue and Ogura⁶ in a completely chemically defined medium when provided with cholesterol (2×10^{-5} M), hematin (0.5 $\mu\text{g/ml}$), lecithin (2 $\mu\text{g/ml}$), and coenzyme Q₁₀ (1 $\mu\text{g/ml}$). The stimulatory effect of coenzyme Q₁₀ has been equivocal, but in the presence of all four compounds, more than 13 serial transfers have been made successfully that involved inoculum levels as low as 20,000 cells per ml. The PK cell line was unique among a number of established cell lines of various species of origin in being unable to synthesize adequate levels of lipids for growth. Therefore, it may serve as a useful tool in future research leading to elucidation of mechanisms of intermediary lipid metabolism.

LITERATURE CITED

1. Bailey, J.M. 1966. Lipid metabolism in cultured cells: VI. Lipid biosynthesis in serum and synthetic growth media. *Biochim. Biophys. Acta* 125:226-236.
2. Sato, G.; Fisher, H.W.; Puck, T.T. 1957. Molecular growth requirements of single mammalian cells. *Science* 126:961-964.
3. Lockart, R.Z.; Eagle, H. 1959. Requirements for growth of single human cells. *Science* 129:252-254.
4. Holmes, R.; Helms, J.; Mercer, G. 1969. Cholesterol requirements of primary diploid human fibroblasts. *J. Cell Biol.* 42:262-271.
5. Inoue, Y.K.; Ogura, R. 1962. Studies and assay of Japanese B encephalitis virus in a stable line of porcine kidney cells. *Virology* 16:205.
6. Oyama, V.I.; Eagle, H. 1956. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocalteu). *Proc. Soc. Exp. Biol. Med.* 91:305-307.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b. GROUP
3. REPORT TITLE		
REQUIREMENTS FOR CHOLESTEROL, HEMATIN, AND LECITHIN FOR OPTIMAL GROWTH OF A PORCINE KIDNEY CELL LINE		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name)		
Kiyoshi (NMI) Higuchi		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
February 1970	13	6
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)	
a. PROJECT NO. 1B562602AD01	Technical Manuscript 591	
c. Task-Work Unit 01-017	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
d. DD 1498 Agency Access. DA OL 0238	CMs 6649 SMUFD-AE-T 49802	
10. DISTRIBUTION STATEMENT		
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
Medical Bacteriology Division		Department of the Army Fort Detrick, Frederick, Md., 21701
13. ABSTRACT		
<p>The nutritional requirements for growth of a porcine kidney (PK) cell line in a chemically defined medium were studied in cells grown as monolayer cultures in T-30 Falcon plastic flasks incubated at 36 C with caps tightened. The PK cell appeared to be unique among a variety of hetero-ploid cell lines in its requirement for a number of unusual substances. Successful propagation of PK cells was obtained in a serum-free defined medium that contained cholesterol (2×10^{-5} M), hematin (0.5 $\mu\text{g/ml}$), lecithin (2 $\mu\text{g/ml}$), and coenzyme, Q_{10} (1 $\mu\text{g/ml}$). The PK cell line may serve as a useful tool in a study of intermediary lipid metabolism at the cellular level.</p>		
14. Key Words		
PK cell line Growth requirements Monolayer cultures Tissue culture Cholesterol		