

UNCLASSIFIED

AD NUMBER
AD842824
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; SEP 1968. Other requests shall be referred to the Army Biological Laboratory, Attn: Technical Release Branch [TID], Fort Detrick, MD 21701.
AUTHORITY
BDRL, per d/a ltr dtd 29 Sep 1971

THIS PAGE IS UNCLASSIFIED

AD

TECHNICAL MANUSCRIPT 479

AD 842824

IMPROVED GROWTH OF MAMMALIAN
AND INSECT CELLS IN CULTURE
WITH HIGH LEVELS OF CHOLINE

Stanley C. Nagle, Jr.

SEPTEMBER 1968

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

RECEIVED
NOV 12 1968
RETURNED

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Releases 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.

STI	WHITE SECTION	<input type="checkbox"/>
OC	DIFF SECTION	<input checked="" type="checkbox"/>
BACKGROUND		<input type="checkbox"/>
JUSTIFICATION		
BY		
DATE OF DISPOSITION AVAILABLE		
DIST.	AVAILABLE	
2		

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 479

IMPROVED GROWTH OF MAMMALIAN AND INSECT CELLS IN CULTURE
WITH HIGH LEVELS OF CHOLINE

Stanley C. Nagle, Jr.

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B527301A082

September 1968

IMPROVED GROWTH OF MAMMALIAN AND INSECT CELLS IN CULTURE
WITH HIGH LEVELS OF CHOLINE

ABSTRACT

Relatively high concentrations of choline chloride produce improved growth of HeLa, L, and Aedes aegypti cells in tissue culture. Approximately 50 mg per liter is optimal for both mammalian and insect cell lines.

Most cell growth media contain about 1 mg per liter of choline, consistent with concentrations usually employed for vitamins. One notable exception is the medium described by Waymouth* containing 250 mg per liter. I observed that, for L cells growing in a chemically defined medium, increased choline permitted continuing growth of cells in the absence of medium replenishment over a longer period of time. Choline levels became deficient, apparently, when it was used at 1 mg per liter. This observation was tested further using HeLa (human), L (mouse), and Aedes aegypti (mosquito) cells.

The mammalian cells were grown in suspension in a heat-stable chemically defined medium as described by Nagle.**

Mosquito cells were grown in suspension in a medium modified from that described earlier.*** The insect cell medium, designated #35, contained the following components in milligrams per liter: L-alanine, 100; L-arginine HCl, 400; L-aspartic acid, 300; L-cysteine HCl, 100; L-glutamic acid, 400; glycine, 400; L-histidine HCl, 200; L-isoleucine, 150; L-leucine, 300; L-lysine HCl, 300; L-methionine, 60; L-phenylalanine, 150; L-proline, 300; L-serine, 150; L-threonine, 75; L-tryptophan, 50; L-tyrosine, 100; L-valine, 150; NaCl, 4,500; KCl, 2,500; CaCl₂·2H₂O, 400; MgCl₂·6H₂O, 400; MgSO₄·7H₂O, 400; NaH₂PO₄·H₂O, 200; glucose, 5,000; D-biotin, 1.0; choline Cl, 1.0 (except where noted); folic acid, 1.0; niacinamide, 1.0; Ca pantothenate, 2.0; pyridoxal HCl, 1.0; thiamine HCl, 1.0; i-inositol, 1.0; riboflavin, 0.1; vitamin B₁₂, 0.002. The medium was adjusted to

* Waymouth, C. 1959. Rapid proliferation of sublines of NCTC clone 929 (strain L) mouse cells in a simple chemically defined medium (MB 752/1). J. Nat. Cancer Inst. 22:1003-1017.

** Nagle, S.C., Jr. 1968. Heat-stable chemically defined medium for growth of animal cells in suspension. Appl. Microbiol. 16:53-55.

*** Nagle, S.C., Jr.; Crothers, W.C.; Hall, N.L. 1967. Growth of moth cells in suspension in hemolymph-free medium. Appl. Microbiol. 15:1497-1498.

pH 6.5 with KOH and sterilized by autoclaving at 121 C for 15 minutes. After cooling, 15 centipoises methylcellulose (as a 2% sterile solution) was added to a final concentration of 0.05%, L-glutamine was added (500 mg per liter) as a dry powder after autoclaving in a stoppered 15-ml serum bottle for 1 hour at 121 C, heated fetal bovine serum (15 minutes at 56 C) was added to a final concentration of 5%, and streptomycin and penicillin were added at final concentrations of 100 μ g and 100 units per ml, respectively.

Cultures of HeLa and L cells were grown at 34 to 36 C in rubber-stoppered 100-ml serum bottles, containing 25 ml of medium, on a New Brunswick Gyrotory shaker* operating at 124 to 130 rpm. Mosquito cells were grown at 26 to 28 C in 250-ml Falcon plastic flasks,** also containing 25 ml of medium, on a New Brunswick Gyrotory shaker operating at 60 rpm. Cell numbers were determined by hemacytometer count. Viability of mammalian cells was routinely observed by counting only those cells with the ability to exclude trypan blue. The trypan blue procedure was not used for mosquito cells; only obviously healthy, intact cells were counted, however.

Results of growth of the three cell lines in their respective media containing graded levels of choline are given in Table 1. Three cultures were prepared for each concentration of choline for each cell line. The data represent the average values for at least two sets of triplicate cultures for each concentration, based on the highest counts obtained in 5, 6, and 7 days of incubation for HeLa, L, and mosquito cells, respectively. Media were not changed during growth. Fifty mg per liter of choline chloride appeared to be optimal for all cells. Cell populations in media with 50 mg per liter compared with those obtained with 1 mg per liter were higher by 28, 143 and 57%, respectively, for HeLa, L, and A. aegypti cells. Growth of A. aegypti cells in the absence of added choline is explained by the fact that the growth medium for these cells contained 5% serum.

Daily replacement of media with fresh media containing 50 mg of choline per liter resulted in a yield of 32.7×10^5 HeLa cells per ml in 9 days and a yield of 59.8×10^5 L cells per ml in 8 days. These values are about equal to (HeLa) or better than (L) populations reported earlier*** and were obtained 1 day (HeLa) or 2 days (L) sooner. A. aegypti cells reached populations of only 10.6×10^5 per ml in 9 days under these conditions. Comparison of these figures with data given in Table 1 shows that the chemically defined medium for HeLa and L cells may become depleted of nutrients when not replenished or that growth is limited by accumulation of certain inhibitory metabolites. Limitation of growth of A. aegypti cells probably was not due to depletion of nutrients because medium changes were not beneficial.

* New Brunswick Scientific Co., Inc., New Brunswick, N.J. 08903.

** Falcon Plastics Division of B-D Laboratories, Inc., Los Angeles, Calif. 90045.

*** Nagle, S.C., Jr. 1968 Heat-stable chemically defined medium for growth of animal cells in suspension. Appl. Microbiol. 16:53-55.

TABLE 1. EFFECTS OF CHOLINE ON GROWTH OF HeLa,
L, AND AEDES AEGYPTI CELLS

Choline Cl Concn, mg/liter	No. of Cells per ml, x 10 ^{5a} /		
	HeLa	L	<u>A. aegypti</u>
0	2.2	3.0	9.7
1	6.1	6.1	10.9
10	6.7	11.4	15.8
50	7.8	14.8	17.1
100	6.1	14.7	16.0
200	5.0	14.1	12.1

a. Zero-time populations were 2×10^5 to 3×10^5
per ml for all cell types.

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) Department of the Army Fort Detrick, Frederick, Maryland, 21701		2a. REPORT SECURITY CLASSIFICATION Unclassified
		2b. GROUP
3. REPORT TITLE IMPROVED GROWTH OF MAMMALIAN AND INSECT CELLS IN CULTURE WITH HIGH LEVELS OF CHOLINE		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name) Stanley C. Nagle, Jr.		
6. REPORT DATE September 1968	7a. TOTAL NO. OF PAGES 9	7b. NO. OF REFS 3
8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) Technical Manuscript 479	
b. PROJECT NO. IB522301A082	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
c.		
d.		
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 Department of the Army Fort Detrick, Frederick, Maryland, 21701	
13. ABSTRACT Relatively high concentrations of choline chloride produce improved growth of HeLa, L, and <u>Aedes aegypti</u> cells in tissue culture. Approximately 50 mg per liter is optimal for both mammalian and insect cell lines.		
14. Key Words Choline chloride HeLa cells <u>Aedes aegypti</u> Tissue culture L cells Nutrition		