Description of document:	Defense Intelligence Agency report, Tissue Culture Technology (BW-Related): USSR, August 1969
Requested date:	23-October-2008
Released date:	10-June-2013
Posted date:	13-August-2013
Source of document:	Commander US Army Intelligence & Security Command Freedom of Information/Privacy Office ATTN: IAMG-C-FOI 4552 Pike Road Fort George G. Meade, MD 20755-5995 Fax: (301) 677-2956 Email: FOIA/Privacy Office Online FOIA Request Form
Note:	This report is one of 16 reports released under Mandatory Declassification Review by the US Army Intelligence & Security Command. All of these reports may be accessed here: http://www/governmentattic.org/inscomBWCW.html

DEPARTMENT OF THE ARMY
UNITED STATES ARMY INTELLIGENCE AND SECURITY COMMAND
FREEDOM OF INFORMATION/PRIVACY OFFICE
FORT GEORGE G. MEADE, MARYLAND 20755-5995

REPLY TO
ATTENTION OF:

Freedom of Information/
Privacy Office

10 JUN 2013

This is in further response to your Freedom of Information Act (FOIA) request of October 23, 2008, and supplements our electronic message of May 12, 2010.

Coordination has been completed with another element of our command and other government agencies and records returned to this office for our review and direct response to you. We have reviewed the records and determined the records are partially releaseable to you. A copy of the records are enclosed for your use.

We have completed a mandatory declassification review in accordance with Executive Order (EO) 13526. As a result of our review information has been sanitized and 4 pages have been withheld in their entirety as the information is currently and properly classified TOP SECRET, SECRET and CONFIDENTIAL according to Sections 1.2(a)(1), 1.2(a)(2), 1.2(a)(3) and 1.4(c) of EO 13526. This information is exempt from the public disclosure provisions of the FOIA pursuant to Title 5 U.S. Code 552 (b)(1). It is not possible to reasonably segregate meaningful portions of the withheld pages for release. The records are enclosed for your use. A brief explanation of the applicable sections follows:

Section 1.2(a)(1) of EO 13526, provides that information shall be classified TOP SECRET if its unauthorized disclosure reasonably could be expected to cause exceptionally grave damage to the national security.

Section 1.2(a)(2) of EO 13526, provides that information shall be classified SECRET if its unauthorized disclosure reasonably could be expected to cause serious damage to the national security.

Section 1.2(a)(3) of EO 13526, provides that information shall be classified CONFIDENTIAL if its unauthorized disclosure reasonably could be expected to cause serious damage to the national security.

Section 1.4(c) of EO 13526, provides that information pertaining to intelligence activities, intelligence sources or methods, and cryptologic information shall be considered for classification protection.

In addition, information has been sanitized from the records and 4 pages have been withheld in their entirety as the release of the information would reveal sensitive intelligence methods. This information is exempt from public disclosure pursuant to Title 5 U.S. Code 552 (b)(7)(E) of the FOIA. The significant and legitimate governmental purpose to be served by withholding is that a viable and effective intelligence investigative capability is dependent upon protection of sensitive investigative methodologies. It is not possible to reasonably segregate meaningful portions of the withheld pages for release.

The withholding of the information described above is a partial denial of your request. This denial is made on behalf of Major General Stephen G. Fogarty, the Commanding General, U.S. Army Intelligence and Security Command, who is the Initial Denial Authority for Army intelligence investigative and security records under the FOIA. You have the right to appeal this decision to the Secretary of the Army. Your appeal must be postmarked no later than 60 calendar days from the date of this letter. After the 60-day period, the case may be considered closed; however, such closure does not preclude you from filing litigation in the courts. You should state the basis of your disagreement with the response and provide justification for a reconsideration of the denial. An appeal may not serve as a request for additional or new information. An appeal may only address information denied in this response. Your appeal is to be made to this office, for forwarding, as appropriate to the Secretary of the Army, Office of the General Counsel.

Coordination has been completed and we have been informed by the Central Intelligence Agency (CIA) that information is exempt from public disclosure pursuant to Title 5 U.S. Code 552 (b)(1) and (b)(3) of the FOIA.

The withholding of the information by the CIA constitutes a denial of your request and you have the right to appeal this decision to the Agency Release Panel within 45 days from the date of this letter. If you decide to file an appeal, it should be forwarded to this office and we will coordinate with the CIA on your behalf. Please cite CIA #F-2010-01292/Army #57F-09 assigned to your request so that it may be easily identified.

Coordination has been completed and we have been informed by the Defense Intelligence Agency (DIA) that their information is exempt from public disclosure pursuant to Title 5 U.S. Code § 552 (b)(1), (b)(2) (b)(3) and (b)(4) of the Freedom of Information Act and Executive Order (EO) 13,526 § 1.4 (c) (d) and (h). The statute invoked under Title 5 U.S. Code 552 (b)(3) is 10 U.S.C. §424, which allows for the protection of organizational and personnel information for DIA.

The withholding of the information by the DIA constitutes a partial denial of your request and you have the right to appeal this decision directly to the DIA. If you decide to file an appeal, it should be forwarded to the Director, Defense Intelligence Agency, ATTN: DAN-1A-FOIA, Washington, DC 20340-5100. Please cite MDR #0155-2010 assigned to your request so that it may be easily identified.

You have received all Army intelligence investigative records pertaining to this request.

There are no assessable FOIA fees.

If you have any questions regarding this action, feel free to contact this office at 1-866-548-5651, or email the INSCOM FOIA office at: INSCOM_FOIA_ServiceCenter@mi.army.mil and refer to case #57F-09.

Sincerely,

Brad S. Dorris
Director
Freedom of Information/Privacy Office
Investigative Records Repository

Enclosure

FSTC-CS-03-7-68 INT

DEFENSE INTELLIGENCE AGENCY

P&D

IA77-55413

501-04 Tissue Cultures

TISSUE CULTURE TECHNOLOGY (BW-RELATED): USSR (U)

ST-S-3-4334

Cy 13 of [illegible]

CONFIDENTIAL SECRET

August 1969

FSTC-CS-03-7A-68-INT

Publication No.
FSTC-CS-03-7-68-INT
Amendment A

US ARMY MATERIEL COMMAND
FOREIGN SCIENCE AND TECHNOLOGY CENTER
Munitions Building, Washington, D.C. 20315

TISSUE CULTURE TECHNOLOGY (BW-RELATED): USSR (U)

Publication No. FSTC-CS-03-7-68-INT, July 1968, is amended as follows:

1. (U) The overall classification of FSTC-CS-03-7-68-INT is changed from SECRET Group 3 to CONFIDENTIAL Group 3. Necessary pen-and-ink changes will be made to accomplish the downgrading.

2. (U) Make the following pen-and-ink changes:

(U) Page vi, LIST OF ILLUSTRATIONS: Before "Diagram . . ." insert "1."

Add

"2. Tissue Culture Vessel ----------16.1"

(U) Page vii, first para, line 3: Change "medical" to "public health".

(U) Page viii, first para, line 1: Change classification from "(U)" to "(C)".

(b)(1)

(U) Page 13, para 1b, line 3: Change "VNK-21" to BHK-21".

(U) Page 19, para 4d, line 2: Delete "?"

(C) Page 20: Add the following paragraph:

(b)(1)

3. (U) Remove pages 9, 10, 15, 16, 17, 18, 21, and 22 and insert new pages 9, 10, 15, 16, 16.1, 17, 18, 21, and 22.

4. (U) Insert new pages iv.1 and iv.3.

(Reverse Blank)

FSTC-CS-03-7A-68-INT
August 1969

(b)(7)(E)

(Total: 213 copies)

FSTC-CS-03-7A-68-INT
August 1969

LIST OF EFFECTIVE PAGES

FSTC-CS-03-7A-68-INT
August 1969

RECORD OF CHANGES

CHANGE NUMBER	DATE OF CHANGE	DATE ENTERED	SIGNATURE RANK/RATE AND ORGANIZATION OF INDIVIDUAL ENTERING CHANGE
A	August 1969		

(Reverse Blank)

TISSUE CELL STRAINS (Continued)

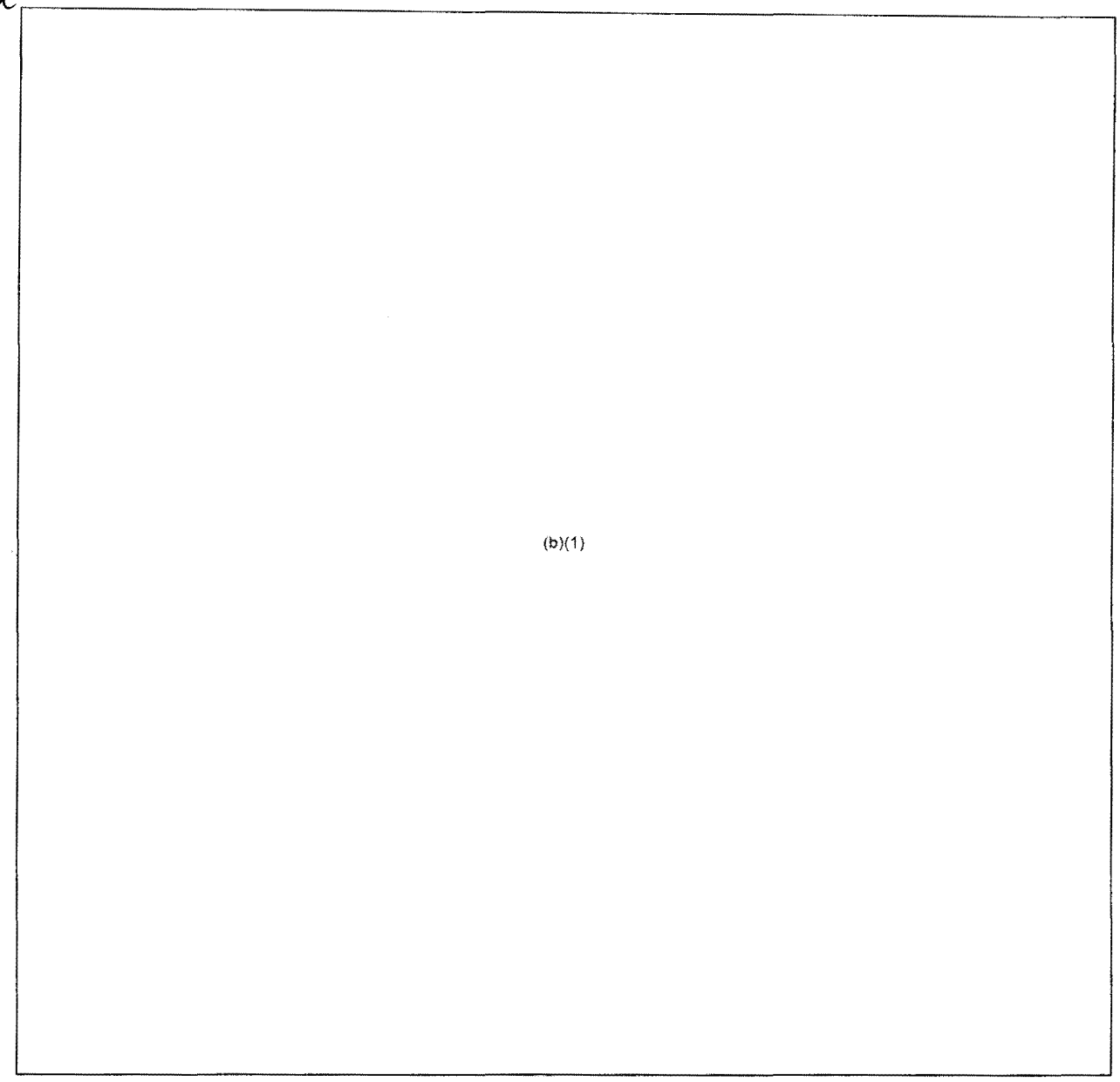
Cell line (continued)	Tissue source (continued)
ERK-1	Rabbit kidney
HEP-2	Human larynx
RES	Fetal pig kidney
CEC	Chick embryo cells
BHK-21	Syrian hamster kidney
WI-38	Human embryo (diploid)
Detroit-6	Human embryo (diploid)
FL	Human amnion
PKP	Lamb kidney
SK	Human lung
SPE	Swine kidney
S-44	Human diploid

Tissue cell isolates from a number of different sources include the following:

Human embryo kidney	Puppy testis
Human embryo skin	Rabbit kidney
Human embryo lung	Rabbit testis
Sheep embryo kidney	Guinea pig kidney
Monkey kidney	Guinea pig testis
Monkey testis	Swine embryo
Puppy kidney	Hamster kidney

b. (U) The classic methods, or variations thereof, were used to obtain, for cultivation, dispersed cells from human and animal tissues. Cell suspensions were counted in the Garyaeva chamber before being planted in Rous or Pavitsky bottles. After cells grew on the glass, the standard procedures for maintaining the established cell lines were followed.

c. (U) In recent years, the Soviets have been considerably interested in the cultivation and use of human diploid cells. Dr. R. I. Rapoport, MNIIVP, isolated 13 strains of diploid cells from human embryo lung tissue and determined the stability of the diploid cells in various media. A long-term study of one line (L-10) showed that the best results were obtained with Eagle's medium No. IX, which contained 10% bovine serum. The diploid complement of chromosomes was preserved, and the line was devoid of oncogenic (tumor-producing) effects. The L-10 cell line was also sensitive to measles, tick-borne encephalitis (TBE), and adenoviruses.



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Original

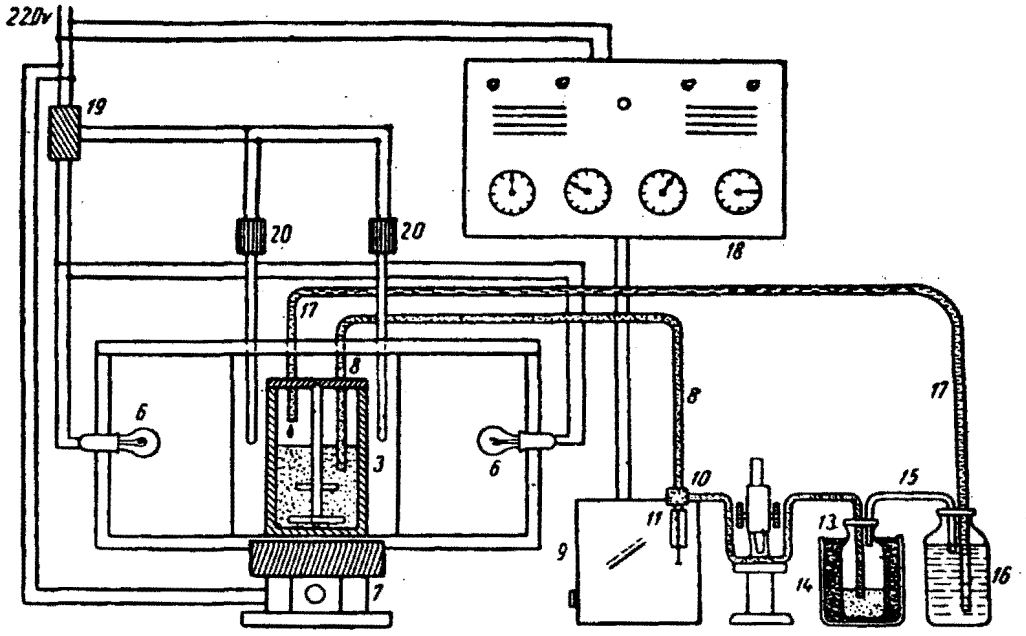

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Figure 1. Diagram of design of a simple hemostat for continuous cultivation of tissue cultures (author's modification) (U).

1 and 2 (missing).
3, plastic reactor.
4 and 5 (missing).
6, heater.
7, magnetic agitator.
8, polyvinylchloride discharge tube.
9, pouring machine or peristaltic pump.
10, valve adapter.
11, injector.
12, device for microscopic inspection of growing cells.
13, receiving flask.
14, container for ice.
15, connecting pipe.
16, flask with extra nutrient medium.
17, polyvinylchloride feeding tube.
18, electronic device regulating feeding of fresh nutrient medium.
19, thermorelay.
20, contact thermometers.

c. (U) Special nutrient mixtures were used to maintain the viability of cells cultivated in suspension. Nutrient media were supplemented by the addition of amino acids, vitamins, glucose, lactalbumin hydrolysate, and other ingredients. The composition of Pirt and Thackeray nutrient medium for the growth of rabbit kidney cells was cited as an example of medium used for suspended cell cultures. The constitutents of the medium were:

Ingredient	Mg/liter	Ingredient	Mg/liter
Lactalbumin hydrolysate	5000	Hypoxanthine	10.0
l-glutamine	100	Vitamin B12	1.0
l-glutamic acid	300	NaCl	3810
dl-methionine	100	KCl	351
l-arginine hydrochloride	250	$CaCl_2$	300
Inositol	.4	$MgCl_2.6H_2O$	200
Biotin	.4	$NaHCO_3$	2500
Choline	1.2	NaH_2PO_4	500
Folic acid	.04	Phenol red	10
Calcium pantothenate	1.2	Glucose	2500
Niacinamide	1.2	Carboxymethyl cellulose	1000
Pyridoxine hydrochloride	.2	Bovine serum (%)	5
Thiamine	2.0	Neomycin units/liter	100,000
Riboflavin	.2		
Demineralized water (ml) Up to 1000			

d. (U) To eliminate cell aggregation in suspended cultures, methyl cellulose, darvan, "pluronik," bactopeptone, and polysulfonic acid were added. Introduction of these components increased the yield of viable cells. Other ingredients, such as insulin, protamine sulfate, and aspergillin-o stimulated the multiplication of inoculated cells.

e. (U) A semiautomatic system for propagation of tissue cells was described by L. N. Mishin, Institute of Virology imeni Ivanovskiy (see figure 2). The concentration of air or CO_2 in a gas mixture was automatically adjusted to maintain a preset level of CO_2 in a sodium bicarbonate-buffered medium. Mixing was provided by a float-type blade mounted inside the culture vessel and driven by a magnetic stirrer. The use of a magnetically driven blade eliminated the need for a shaft seal and reduced contamination problems. The apparatus, operated semi-continuously for several months, maintained good culture conditions. Infectivity studies using VEE virus and chick embryo fibroblast cells showed that the device could be used to propagate viruses. A titer of 2.1×10^9 virus particles per milliliter was obtained at a cell concentration of 2×10^6 cells per milliliter.

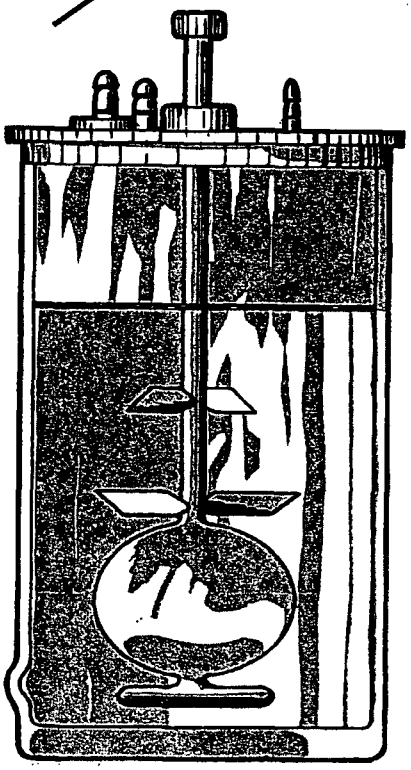

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Figure 2. Tissue culture vessel (U).

f. (U) Work on automated tissue cell culture systems indicates that the Soviets have successfully copied techniques previously reported and have incorporated modifications which contribute to the simplicity and dependability of operation. The method of continuous cell cultivation is well suited to the industrial production of cell cultures, antigens, viruses, and nucleic acids in mass quantities. The method is more advantageous than ordinary systems for producing viruses and may make it possible to obtain large concentrations of viruses for the solution of many theoretical and practical problems. In addition, the cultivation of TBE, VEE, and adenoviruses shows much promise.

(b)(1)

Section IV (C)

TISSUE CULTURE IN VIROLOGY

(b)(1)

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3. (U) Diagnosis and Detection of Viruses

a. The application of tissue cell culture techniques for the diagnosis and detection of viruses has been studied extensively by Dr. S. Ya. Gaydamovich's group at the Institute of Virology imeni Ivanovskiy. Diagnosticums for the complement fixation reaction and for inhibition of hemagglutination with TBE, VEE, WEE, and Japanese B encephalitis have been prepared in tissue cells. In addition, a neutralization reaction based on the titration of excess hemagglutinins of TBE and Japanese B encephalitis has been used successfully.

b. The fluorescent antibody staining technique (FAST) has been applied to the detection of arboviruses in tissue cultures. A method of contrasting nonspecific proteins by using rhodamine dyes with the specific fluorescence of arboviruses has shown greater reliability and specificity than conventional methods for the detection of viruses. R. M. Gol'din, Military Medical Academy imeni Kirov, found that the addition of red fluorescing rhodamine dyes was quite effective in suppressing nonspecific fluorescence. Pronounced contrasting was obtained by staining nonspecific proteins with sulforhodamine B. Although the background was stained red, the coloring did not suppress the bright green specific fluorescence of the viruses.

(b)(1)

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Section V (A)

TRENDS IN TISSUE CULTURE TECHNIQUES

1. (C) Past

(b)(1)

2. (U) Present

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3. (U) Future

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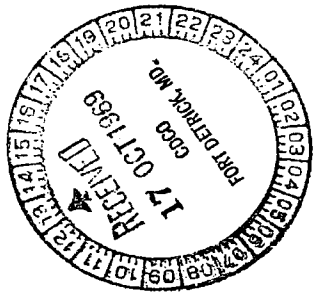

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TISSUE CULTURE TECHNOLOGY (BW-RELATED): USSR (U)

FSTC-CS-03-7-68-INT

DIA TASK T67-03-6

July 1968

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PREFACE

(b)(1)

(U) Although the cutoff date for information in this document is March 1968, major updatings were made to the date of final approval for printing.

(U) This study has been prepared with the editorial assistance of (b)(6) (b)(6)

(U) Comments concerning the study may be forwarded to the Commanding Officer, Attn: AMXST-AB, US Army Foreign Science and Technology Center, Munitions Building, Washington, D. C. 20315.

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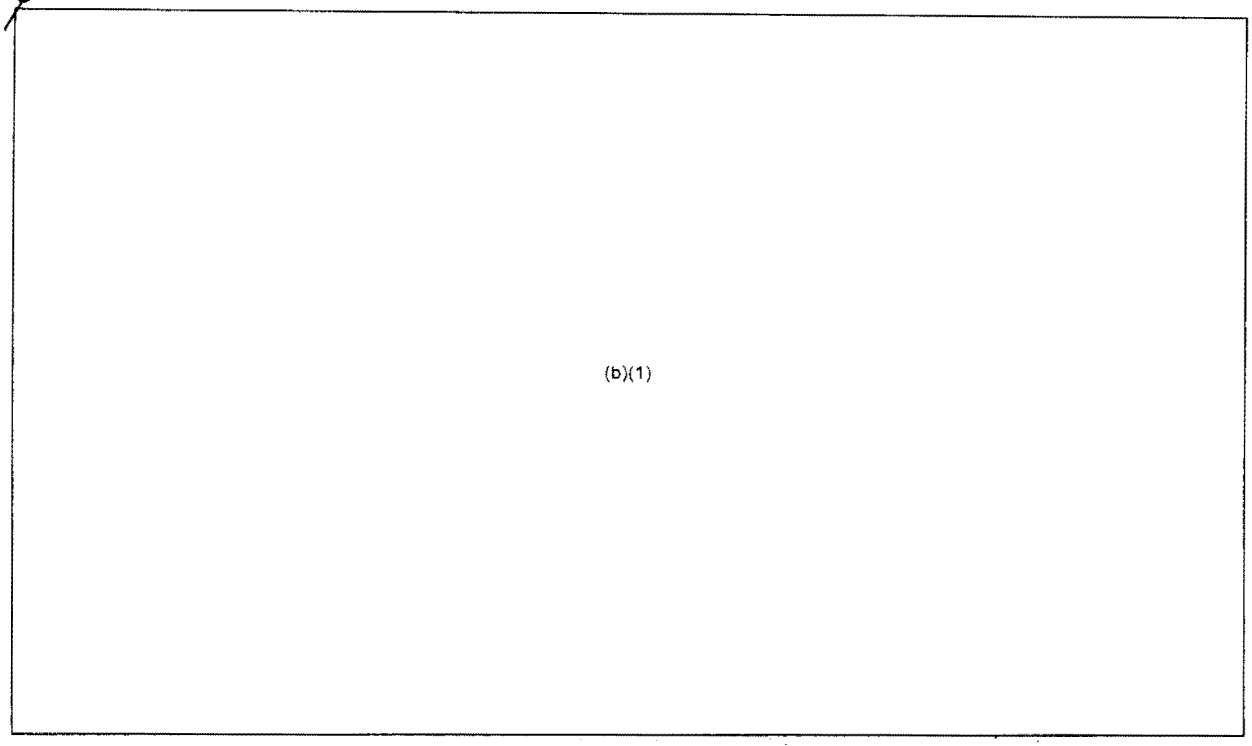
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Original

Section I (C).

TISSUE CELL CULTURE APPLICATIONS

1. (C) General

(b)(1)

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Section II (S).

TISSUE CULTURE TECHNIQUES

1. (C) Tissue Culture Media

a. (U) Soviet scientists have explored the growth of cells isolated from many human and animal sources in different media and under different growth conditions. Through extensive literature surveys and applied research, they have determined that tissue cells demand a minimum of 12 amino acids, eight vitamins, six inorganic ions, glucose, and serum for growth in vitro. These basic-medium components are shown in table I.

Table I. Minimum Nutritive Requirements for Cells in Tissue Cultures (U)

Amino acids	Carbohydrates	Vitamins and factors	Ions	Protein
Leucine -------	Glucose[1] --	Choline -------	Sodium -----	Serum
Isoleucine -----	----------	Folic acid ----	Potassium --	------
Lysine -------	----------	Pantothenate --	Calcium ----	------
Methionine ----	----------	Pyridoxol -----	Magnesium --	------
Phenylalanine --	----------	Riboflavin ----	Chlorides ---	------
Tryptophan ---	----------	Thiamine -----	Phosphates --	------
Threonine ----	----------	Inositol ------	----------	------
Valine -------	----------	Nicotinamide --	----------	------
Arginine -----	----------	----------	----------	------
Tyrosine -------	----------	----------	----------	------
Histidine ------	----------	----------	----------	------
Glutamine -----	----------	----------	----------	------
Cystine[2]	----------	----------	----------	------
Glycine[3] -------	----------	----------	----------	------

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[1] Fructose, mannose, galactose, etc, also can be used.
[2] Not needed in presence of restoring inorganic serum compounds in the medium.
[3] Needed for primary cultures of cells of the monkey kidney.

b. (U) The addition of supplementary ingredients to the basal medium stimulated the multiplication of cells at an optimum pH range between 7.2 and 7.4. A number of synthetic media, including medium No. 199 and Eagle's and Earle's media, were explored by (b)(6) Institute of Epidemiology and Microbiology imeni Gamaleya, Moscow, and (b)(6) Moscow Scientific Research Institute for Virus Preparations (MNIIVP). Medium No. 199 with some modifications (table II), used extensively in Soviet laboratories, is prepared in a single-strength and a tenfold concentrate at the MNIIVP and distributed to the using laboratories. Eagle's medium, trypsin, balanced salt solution, serum, and other tissue culture reagents also are produced. Because of the known composition, the synthetic media are important for the study of viral infectivity and the cell-virus interrelationships, for biosynthesis within the cell, and for the preparation of vaccines.

Table II. The No. 199 Medium (U).

Composition	Mg/liter
Inorganic salts:	
Sodium chloride (NaCl)	8000
Potassium chloride (KCl)	400
Calcium chloride ($CaCl_2$)	1400
Magnesium sulfate ($MgSO_4.7H_2O$)	200
Sodium hydrophosphate ($Na_2HPO_4.2H_2O$)	60
Potassium hydrophosphate (KH_2PO_4)	60
Sodium bicarbonate ($NaHCO_3$)	1500
Iron nitrate ($Fe(NO_3)_3.9H_2O$)	100
Amino acids:	
l-arginine monohydrochloride	70
l-histidine monohydrochloride	20
l-lysine monohydrochloride	70
dl-tryptophan	20
dl-phenylalanine	50
dl-methionine	30
dl-serine	50
dl-threonine	60
dl-leucine	120
dl-isoleucine	40
dl-valine	50

Table II. The No. 199 Medium (U). (Continued)

Composition	Mg/liter
Amino acids: (continued)	
dl-glutamic acid monohydrate	150
dl-asparaginic acid	60
dl-alpha-alanine	50
l proline	40
oxy-l-proline	10
glycine	50
l-histidine	20
l-tyrosine	40
l-cysteine hydrochloride	.1
Vitamins:	
Nicotinic acid (niacin)	.025
Nicotinamide (niacinamide)	.025
Pyridoxine hydrochloride	.025
Thiamine hydrochloride	.010
Pyridoxol hydrochloride	.025
Riboflavin	.010
Calcium pantothenate	.010
L-inositol	.050
P-aminobenzoic acid	.050
Choline chloride	.500
D-biotin	.01
Folic acid (crystalline)	.01
Calciferol (vitamin D_2)	.1
Alpha-tocopherol sodium phosphate (vitamin E)	.01
Menadione (2-methyl-1, 4-naphthoquinone) (vitamin K_3)	.01
Crystalline vitamin A	.1
Ascorbic acid (vitamin C)	.05
Lipoid sources:	
Tween 80	20
Cholesterin	.2

Table II. The No. 199 Medium (U). (Continued)

Composition	Mg/liter
Nuclenic acid components:	
Adenine sulfate	10
Xanthine	.3
Hypoxanthine	.3
Thymine	.3
Uracil	.3
Guanine hydrochloride	.3
Adenosine triphosphate sodium	10.0
Adenylic acid	.2
D-ribose	.5
D-desoxyribose	.5
Other substances:	
Sodium acetate	50
l-glutathione	.05
Glucose	1000
l-glutamine	100
Phenol red	20
Ethyl alcohol	16
Antibiotics:	
Penicillin (sodium salt) (units)	100,000
Streptomycin (chlor-calcium complex) (micrograms)	100,000

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NOTE: Method of preparation--Preparation of the No. 199 medium is carried out in three stages:
(a) Preparation of the basic concentrated solutions of the number of ingredients.
(b) Preparation of a tenfold concentrate of the medium.
(c) Preparation of the operating dilution of medium No. 199.
For the preparation of all solutions, bisdistilled water, which is obtained in glass apparatuses, is used.

c. (U) Soviet researchers have explored the nutritional value of the hydrolysates of natural products: casein, lactalbumin, and whole-blood protein (Leibenson), which have been labeled "amino-peptide-2." Amino-peptide-2 is manufactured at the Kirov Factory of Medicinal Preparations of the Leningrad Meat Combine. A medium prepared from meat by the same factory has been reported. Supplemented with additional ingredients, these proteins provide high cell proliferation. The composition of several complex media used by the Soviet investigators is shown in table III.

Table III. Comparative Composition of Media With Fermentative Protein Hydrolysates (gm/liter) (U)

Ingredients	Lactalbumin hydrolysate	Lepine's medium	Leibenson's medium	Levrov's medium	Medium of Smirnova and Ermakova
Lactalbumin hydrolysate-	5.0	---	---	---	---
Casein hydrolysate -----	---	0.5	---	---	1.1
Amino-peptide-2 (ml) ---	---	---	300	50-70	---
Serum (ml) ----------	20	25-50	---	100	25
Glutamine ----------	---	0.1	---	---	.1
Cysteine hydrochloride -	---	.01	---	---	.02
Niacin ----------	---	.001	---	1.0	---
Pyridoxine ----------	---	.001	---	1.0	---
Thiamine ----------	---	.001	---	1.0	---
Riboflavin ----------	---	.0001	---	.1	---
Calcium pantothenate ---	---	.001	---	1.0	---
Para-aminobenzoic acid -	---	.001	---	---	---
Choline ----------	---	.001	---	.01	---
Biotin ----------	---	.001	---	---	---
Folic acid ----------	---	.001	---	.01	---
Alpha-tocopherol -------	---	.001	---	---	0.001
Ascorbic acid ----------	---	.025	---	---	.1
Sodium nucleinic acid --	---	---	---	.02	---
Adenosine triphosphoric acid (1%) (ml)	---	---	5	---	---
Earle's solution (ml) --	---	1000	---	---	1000
Hank's solution (ml) ---	1000	---	700	830-850	---

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(b)(1)

2. (c) Tissue Culture Methods

(b)(1)

3. (5) Cell Lines and Cultivation

a. (U) Soviet scientists have employed many of the established cell lines and have initiated many other cell cultures for virus investigations. Some of the cell lines and tissue sources are shown below:

(b)(1)

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(b)(1)

b. (U) The classic methods, or variations thereof, were used to obtain, for cultivation, dispersed cells from human and animal tissues. Cell suspensions were counted in the Garyaeva chamber before being planted in Rous or Pavitsky bottles. After cells grew on the glass, the standard procedures for maintaining the established cell lines were followed.

c. (U) In recent years, the Soviets have been considerably interested in the cultivation and use of human diploid cells. (b)(6) MNIIVP, isolated 13 strains of diploid cells from human embryo lung tissue and determined the stability of the diploid cells in various media. A long-term study of one line (L-10) showed that the best results were obtained with Eagle's medium No. IX, which contained 10% bovine serum. The diploid complement of chromosomes was preserved, and the line was devoid of oncogenic (tumor-producing) effects. The L-10 cell line was also sensitive to measles, tick-borne encephalitis (TBE), and adenoviruses.

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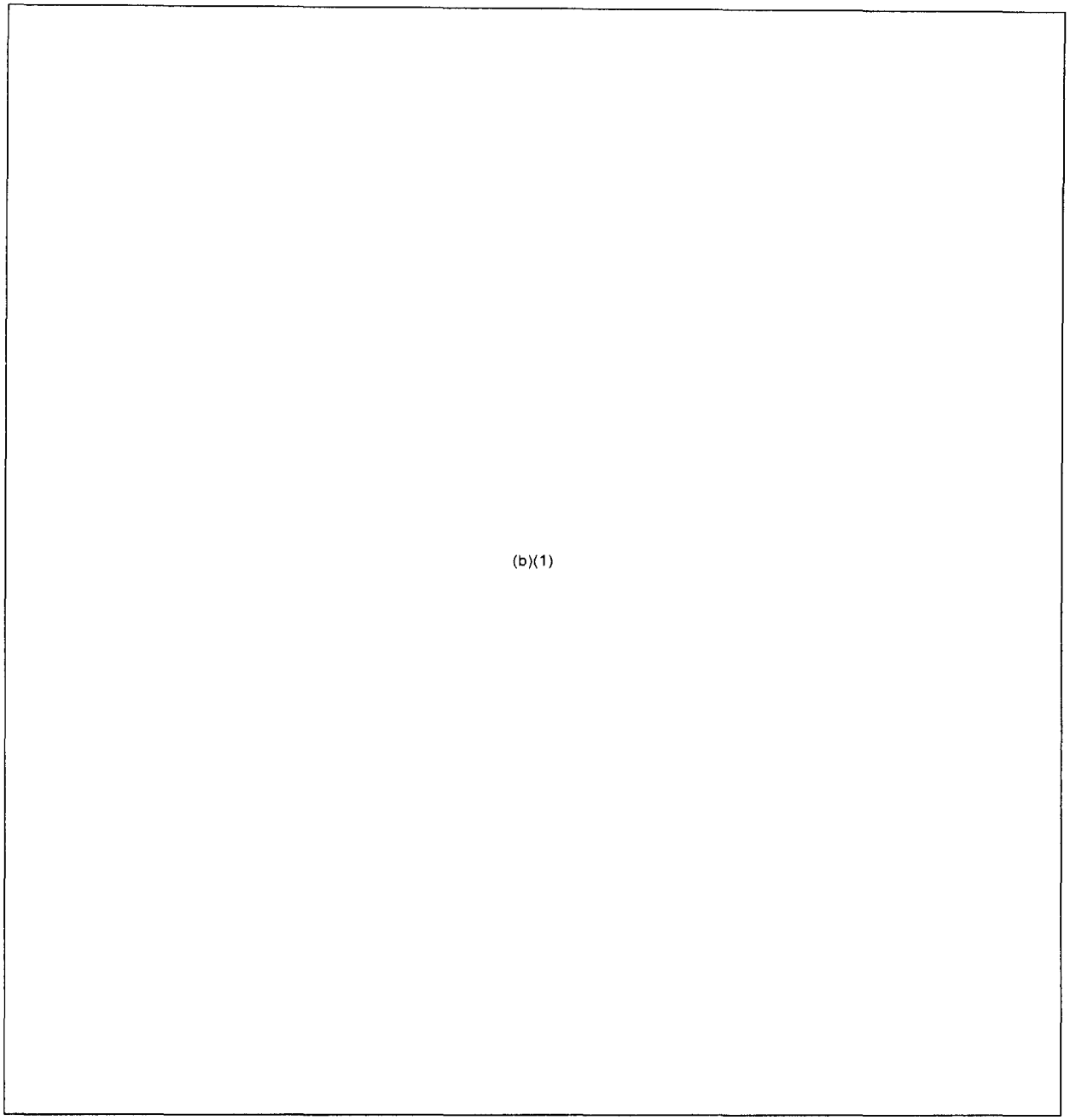

(b)(1)

g. (U) The intensive interest shown by Soviet researchers and the wide distribution of work with human diploid cells indicate a concentrated effort to thoroughly explore the use of diploid cells in virology.

Section III (C)

MASS PRODUCTION METHODS

1. (U) Monolayer Cultures

Soviet researchers have employed monolayer tissue cell cultures to produce vaccines, and the method could be used to grow cells for the production of viral agents. The system is impractical, however, because the contents of large numbers of flasks must be pooled, and the procedure is cumbersome, time consuming, and laden with contamination problems.

2. (U) Roller-Bottle Method

(b)(1)

b. (U) Later, (b)(6) Institute of Virology imeni Ivanovskiy, used a similar system to produce rabies virus in Syrian hamster kidney fibroblasts (BHK-21 cell line). Bottles with capacities ranging from 1 to 20 liters were used, with the volume of medium being about one-twentieth that of the bottle capacity. Inocula were added to give an initial concentration of 4 to 5 X 10^5 cells per milliliter. A special rack with different-sized rollers was designed to rotate bottles, regardless of size, at 0.7 to 0.8 rpm. Cells began to attach to the glass surface 2 hours after inoculation. Most of the cells were attached in 12 to 18 hours, and a complete monolayer was formed after 24 to 48 hours.

c. (U) Primary infection of the cells was accomplished after 24 to 48 hours of cultivation by introducing a 10% suspension of brain cells infected with rabies virus at one-fifth the volume of the medium. Subsequent infections were made by subculturing methods or by mixing infected cells with uninfected cells in fresh medium. Experimental results showed that:

(1) The total number of tissue cells in roller-bottle cultures was three times that obtained in stationary cultures.

(2) The multiplicity of infection was truer in roller bottles than in monolayers because better contact was obtained between cells and virus particles.

(3) During the cultivation of fixed rabies virus in roller bottles by sub-culturing and cell mixing, the virus could be maintained up to the 30th passage as compared with 7 or 8 passages in monolayer cultures.

(4) Maximum titers of $10^{8.8}$ mouse intracranial median lethal dose ($MICLD_{50}$) per ml were obtained on the third day in roller bottles as compared with maximum titers of $10^{8.43}$ $MICLD_{50}$ per ml on the fifth day in stationary cultures.

(5) Cytoplasmic inclusions and CPE's occurred in roller-bottle cultures, and a more regular and more extensive accumulation of specific antigen of rabies virus was detected.

(b)(1)

3. (C) Suspension Cell Cultures

(b)(1)

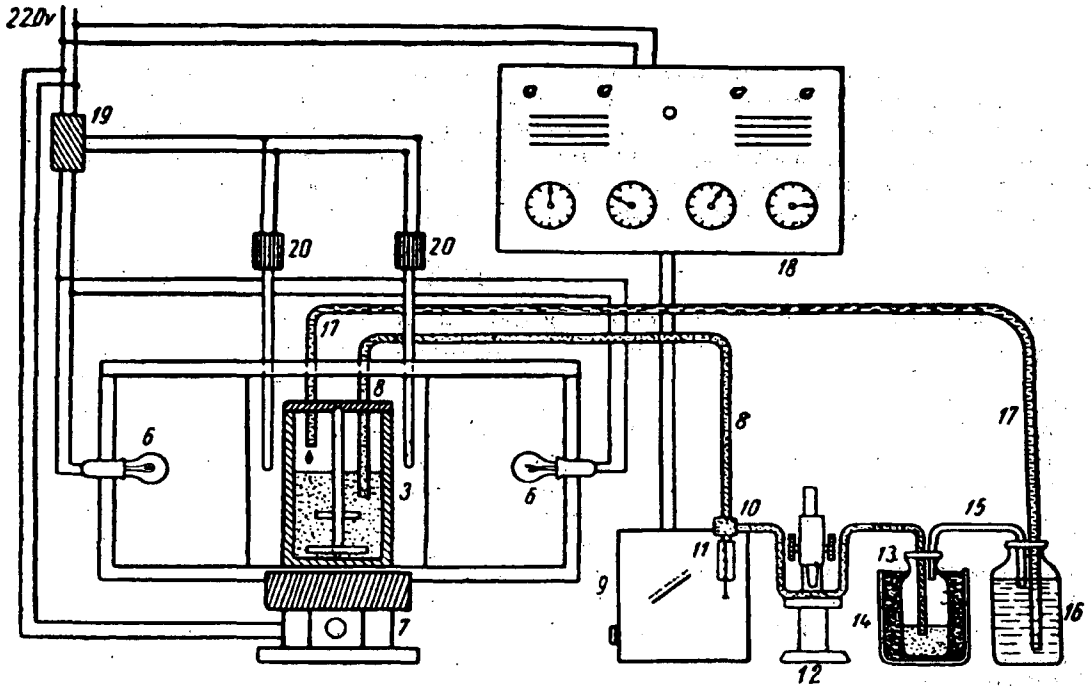

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Diagram of design of a simple hemostat for continuous cultivation of tissue cultures (author's modification) (U)

1 and 2 (missing).
3, plastic reactor
4 and 5 (missing).
6, heater
7, magnetic agitator.
8, polyvinylchloride discharge tube.
9, pouring machine or peristaltic pump.
10, valve adapter.
11, injector.
12, device for microscopic inspection of growing cells.
13, receiving flask.
14, container for ice.
15, connecting pipe.
16, flask with extra nutrient medium.
17, polyvinylchloride feeding tube.
18, electronic device regulating feeding of fresh nutrient medium.
19, thermorelay.
20, contact thermometers.

c. (U) Special nutrient mixtures were used to maintain the viability of cells cultivated in suspension. Nutrient media were supplemented by the addition of amino acids, vitamins, glucose, lactalbumin hydrolysate, and other ingredients. The composition of Pirt and Thackeray nutrient medium for the growth of rabbit kidney cells was cited as an example of medium used for suspended cell cultures. The constitutents of the medium were:

Ingredient	Mg/liter	Ingredient	Mg/liter
Lactalbumin hydrolysate	5000	Hypoxanthine	10.0
l-glutamine	100	Vitamin B_{12}	1.0
l-glutamic acid	300	NaCl	3810
dl-methionine	100	KCl	351
l-arginine hydrochloride	250	$CaCl_2$	300
Inositol	.4	$MgCl_2.6H_2O$	200
Biotin	.4	$NaHCO_3$	2500
Choline	1.2	NaH_2PO_4	500
Folic acid	.04	Phenol red	10
Calcium pantothenate	1.2	Glucose	2500
Niacinamide	1.2	Carboxymethyl cellulose	1000
Pyridoxine hydrochloride	.2	Bovine serum (%)	5
Thiamine	2.0	Neomycin units/liter	100,000
Riboflavin	.2		
Demineralized water (ml)	Up to 1000		

d. (U) To eliminate cell aggregation in suspended cultures, methyl cellulose, darvan, "pluronik," bactopeptone, and polysulfonic acid were added. Introduction of these components increased the yield of viable cells. Other ingredients, such as insulin, protamine sulfate, and aspergillin-o stimulated the multiplication of inoculated cells.

e. (U) The method of continuous cell cultivation is well suited to the industrial production of cell cultures, antigens, viruses, and nucleic acids in mass quantities. The method is more advantageous than ordinary systems for producing viruses and may make it possible to obtain large concentrations of viruses for the solution of many theoretical and practical problems. In addition, the cultivation of TBE, VEE, and adenoviruses shows much promise.

(b)(1)

Section IV (S)

TISSUE CULTURE IN VIROLOGY

1. (S) Viral Reproduction

(b)(1)

2. (S) Plaque Formation

(b)(1)

(b)(1)

3. (U) Diagnosis and Detection of Viruses

a. The application of tissue cell culture techniques for the diagnosis and detection of viruses has been studied extensively by Dr. S. Ya. Gaydamovich's group at the Institute of Virology imeni Ivanovskiy. Diagnosticums for the complement fixation reaction and for inhibition of hemagglutination with TBE, VEE, WEE, and Japanese B encephalitis have been prepared in tissue cells. In addition, a neutralization reaction based on the titration of excess hemagglutinins of TBE and Japanese B encephalitis has been used successfully.

b. The fluorescent antibody staining technique (FAST) has been applied to the detection of arboviruses in tissue cultures. A method of contrasting nonspecific proteins by using rhodamine dyes with the specific fluorescence of arboviruses has shown greater reliability and specificity than conventional methods for the detection of viruses. (b)(6) Military Medical Academy imeni Kirov, found that the addition of red fluorescing rhodamine dyes was quite effective in suppressing nonspecific fluorescence. Pronounced contrasting was obtained by staining nonspecific proteins with sulforhodamine B. Although the background was stained red, the coloring did not suppress the bright green specific fluorescence of the viruses.

(b)(1)

(b)(1)

(b)(1)

5. (U) Interferon

a. Andzhaparidze studied the interference between TBE and WEE in cultures of chick fibroblasts, human diploid cells, HEP-2, HeLa, and transplantable non-malignant cells. The interference phenomenon varied in the IX-10 strains. The interference activity of viruses that were previously adapted to a cell culture increased. The degree of interference was the same for cultures cultivated at 37°C, but was somewhat lower at 29° C.

b. At the Institute of Poliomyelitis and Virus Encephalitis, Shalunova studied the interference of Japanese B encephalitis with poliovirus in human embryo skin cells, and with the viruses of Newcastle disease and WEE in chick embryo cell cultures. Interference of varying degree was noted after 72 hours in cells infected with Japanese B encephalitis. The highest sensitivity was found in chick embryo cells tested with WEE since interference activity titers were close to the virus titers obtained in mice.

6. (S) Vaccine Preparation

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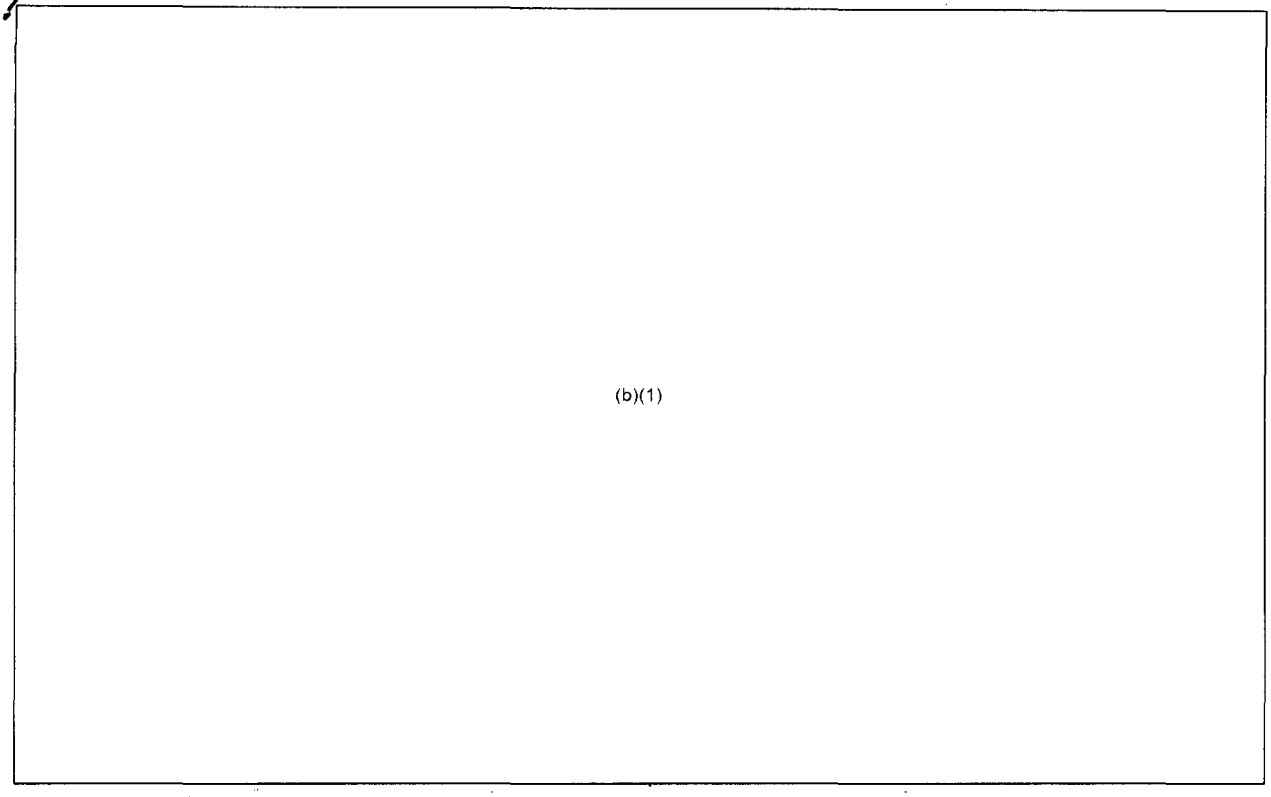

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Section V ~~(S)~~

TRENDS IN TISSUE CULTURE TECHNIQUES

1. ~~(S)~~ Past

(b)(1)

2. ~~(S)~~ Present

(b)(1)

3. ~~(S)~~ Future

(b)(1)

(b)(1)

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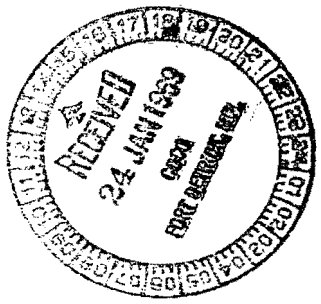

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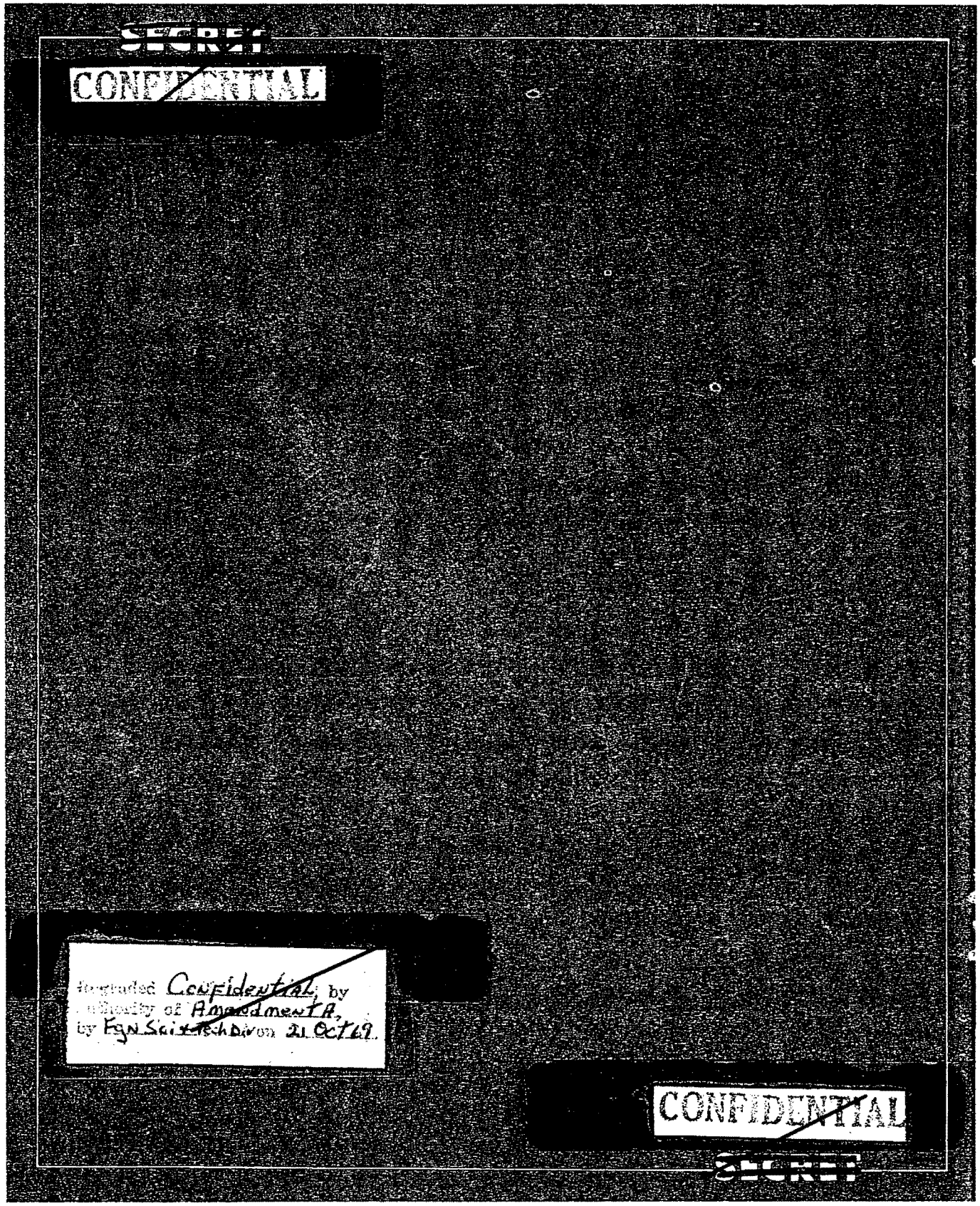
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