

A PROPOSAL TO
International Lead Zinc Research Organization, Inc.

FOR

1. Effect of lead on tissue culture of the nervous system.
IV. Comparative study of the pattern of absorption
of lead, tin, mercury and copper by nervous
tissue in culture.
2. The influence of lead and other heavy metals on the
synthesis and turn-over of RNA in mammalian cells.

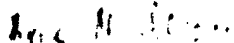
From July 1, 1967 through June 30, 1968

Total Support Requested: \$15,000

Hanna Sobkowitz, M.D., Principal Investigator

Department of Neurology
Medical School

The University of Wisconsin
750 University Avenue
Madison, Wisconsin 53715



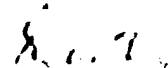
(Signature)

Hanna Sobkowitz, M.D.
Principal Investigator



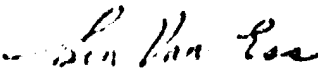
(Signature)

Peter L. Eichman, M.D.
Dean, Medical School



(Signature)

Francis M. Forster, M.D.
Department Chairman



(Signature)

Len Van Een, Director of
Research Administration-Financial

International Lead Zinc Research Organization, Inc.

292 Madison Ave.

New York, N.Y. 10017

APPLICATION FOR RESEARCH GRANT

Date Feb. 27, 1967

Application is hereby made for a grant in the amount of \$ 15,000 for the period from July 1, 1967 through July 1, 1968 inclusive for the purpose of conducting research on: 1.- Effect of lead on tissue culture of the nervous system, IV.- Comparative study of the pattern of absorption of lead, tin, mercury and copper by nervous tissue in culture.

2.- The influence of lead and other heavy metals on the synthesis and turn-over of RNA in mammalian cells.

Name and title of principal investigator: Hanna M. Sobkowicz, M.D.
Assistant Professor of Neurology.

Address, including name of institution, of principal investigator: Tissue Culture Laboratory of the Department of Neurology
University of Wisconsin Medical School, Madison, Wisconsin 53706.

Name and title of financial officer to whom checks should be mailed: Mr. L. Van-Ess, Director of Research Administration-
Financial.

Address, including name of institution, of financial officer: University of Wisconsin, 750 University Ave. Madison, Wis. 53706.

- 2 -

BUDGET FOR THE PERIOD FROM : JULY, 1, 1967 - JULY 1, 1968

Permanent equipment

AMSCO Dualmatic Deioniser	6800.00
AMSCO purometer and Conductivity meter with 24-h graph recording	1700.00
Microscopic equipment (short distance lenses and condensor)	1300.00

Consumable supplies

Glassware	1000.00
Small instruments & misc. Lab. supplies.....	500.00
Photo supply & work	500.00
Chemicals & precursors of nucleic acids.....	800.00
Animals	1000.00

Other expense

Xerox for bibliography use	100.00
Books concerning toxicology & poisonings	200.00
Heavy metals tests	500.00
Travel expenses	600.00

~~Grand~~ TOTAL 15000.00

JUSTIFICATION OF BUDGET

The histochemical part of our project requires extremely pure water. Reagents used to detect heavy metals in a tissue sample are not specific for a given metal, therefore any contamination of water with other metals in the water supply must be avoided. The water in Madison creates a special problem for our research program as its hardness is ranging from 18 - 22 grains (i.e. high calcium and magnesium); special arrangements are required in addition to already existing apparatus: AMSCO Ultra Pure Water Still plus storage tank and Amersil Quartz Bi-Distillation Apparatus. To provide us with the purity of water needed, our engineering department has specified:

- 1.- AMSCO Dualmatic Deionizer which automatically provides a continuous supply of deionized water of 1 megohm-cm specific resistance.
- 2.- A special final control includes Purometer which automatically diverts distillate of less than desired purity (0.1 - 1 megohm - cm) to waste line.

A conductivity Meter with 24 hour graph recording of the distillate delivered.

These items of equipment are essential if we are to be assured of the chemical specificity of our methods.

- 4 -

RESEARCH PLAN

Principle investigator: Hanna M. Sobkowitz, M.D.
Assistant Professor of Neurology,
Tissue Culture Laboratory
of the
Department of Neurology,
University of Wisconsin Medical School

Project assistant: Rachel Monzain, B.A., R.N.
Project Assistant of the Tissue Culture
Laboratory of the Department of Neuro-
logy, University of Wisconsin Medical
School.

1. Effects of lead on tissue culture of the nervous system.
IV. Comparative study of the pattern of absorption of lead,
tin, mercury and copper by nervous tissue in culture.

The principle aim of our research project is to investigate histochemically the cells which absorb tin, mercury and copper as compared to lead in nervous tissue in culture.

It was demonstrated in our previous study that heavy metals can be divided, depending upon the response of cultured nervous tissue, into two groups: 1.- Metals which appear to exert a direct toxic action on the nerve cell (mercury) and 2.- Metals which are not toxic for the nerve cell itself but destroy the supporting tissue (lead, tin and gold). Furthermore on the basis of a histochemical study, we demonstrated the presence of lead in those supporting tissue cells which suffered injury under exposure to the metal. The hypothesis was made that lead

- 5 -

itself has no direct effect on the nerve cell or the myelin sheath in vitro. This may be due to the demonstrated lack of uptake by the nerve cell from their environment. It is also suggested that the excessive absorption of lead by mesenchymal tissue results in the degeneration of this tissue.

In order to prove our theory that the toxicity of a heavy metal is related to its ability to penetrate inside of the cell, we propose to compare the morphology of nervous system cultures exposed to a series of metals with the histochemical determination of the metals' absorption by nervous tissue in vitro.

Tin, mercury and copper have been chosen for our series. Copper is included because of the work of Uzman (1956)*. He found that copper, which is one of the most toxic metals for nervous tissue, is absorbed by myelin sheath and nerve cell nuclei when sections of normal brain are exposed to the metal. The histochemical study will be performed on absorption of two known toxic metals: mercury and copper, and two metals which did not show a direct action against the nerve cell: lead and tin.

* L. Lahut Uzman: Histochemical Localization of Copper with Rubanic Acid. Lab. Invest., 1956, 5:299-305.

2.- The joint project with Dr. Henry Kubinsky, Assistant Professor of Surgery, Division of Neurosurgery, University of Wisconsin, Medical School.
The Influence of Lead and Other Heavy Metals on the Synthesis and Turn-over of RNA in Mammalian Cells.

Introduction: There are several observations on binding of heavy metals to deoxyribo- and ribonucleic acids (DNA and RNA) and the possible biological significance of such altered macromolecules. To the best of our knowledge, however, no thorough work has been done on the influence of heavy metals on the synthesis of RNA in the treated cell. One

can expect that the patterns of RNA synthesis (i.e., the relative rates of synthesis of ribosomal precursors and "messenger" RNA's) as well as the over-all rate of cellular RNA synthesis may be significantly changed in the cell undergoing such treatment: all treatments which might be considered "toxic" or "Stressful" to the cell seem to result in profound alteration of RNA metabolism (ref. 6, 11, 16).

Experimental techniques:

The synthesis of RNA will be studied with mammalian cells in tissue cultures and in living organism.

For initial studies, however, model "S 180" mouse ascites tumor cells in suspension will be used. The informations obtained with the model system of "S 180" ascites cells will be subsequently used for further studies on the metabolism of nerve cells in vivo and in tissue culture.

The synthesis of RNA in these cells will be studied by exposing them to the sterile solutions of salts of heavy metals (or a combination of such salts) followed by a pulse of radioactive precursors of RNA (P^{32} , H^3 or C^{14} labelled uracil or uridine). In control experiments the cells will be treated similarly in media free of heavy metals. RNA will be extracted by routine methods, fractionated and analysed by: chromatography on methyl-esterfied albumin-kieselguhr (MAK) columns; by sedimentation in sucrose gradients; by hybridisation with DNA and by the analysis of nucleotide composition of various RNA fractions.

These technics will allow separation of various kinds of RNA (transfer, messenger and ribosomal) and permit recognition of the patterns in RNA metabolism.

In addition, the fast labeled RNA derived from sub-cellular particles (cell nuclei and mitochondria) will be studied in parallel. The isolation of these organelles will be made by routine methods.

Under these experimental conditions the patterns of RNA synthesis (amounts of radioactive precursors incorporated into various RNA fractions) will furnish the information on the regulatory mechanism. The techniques described will also permit detection of any RNA normally absent in untreated cells, but appearing as a result of the treatment with heavy metals.

An assesment of the usefulness of the acridine - orange staining technique for the above studies will also be made.

Significance of the proposed research:

It may be expected that informations gained from these studies will increase our understanding of molecular events resulting in cell injury and death due to heavy metals intoxication. It will also increase our understanding of the regulatory mechanism within the living cells and organs.

It is also hoped that this information may ultimately help in better pharmacological control of cellular functions.

Relationship of proposed research to the laboratory's long term goals:

These studies will be part of a bigger research program on the regulation of RNA synthesis within animal cells, with special emphasis on RNA synthesis in central nervous system in vivo and in vitro.

BIOGRAPHICAL SKETCH

H.M. Sobkowiec

Hanna M. Sobkowiec, M.D.

Born: 1.1.1931, Warsaw - Poland

Assistant Professor of Neurology, University of Wisconsin Medical
School, Tissue Culture Laboratory of the Department of Neurology,
Chairman: Francis M. Forster, M.D.

Sept. 1, 1966 - to date

Professional experience:

Fellow of National Multiple Sclerosis
Society at Columbia University; Labo-
ratory for Cell Physiology - Dr. M.R.

Murray,

Feb. 1, 1965 - Sept. 1, 1966

Mount Sinai Hospital, N.Y.C.;

Laboratory of Cellular Neurophysiology,

Dr. M.B. Bornstein,

...

Research Fellow at the Dept. of
Neurology (Dr. M. Bender, Mt. Sinai Hospital,
N.Y.C.; Laboratory of Cellular Neurophy-
siology, Dr. M.B. Bornstein.

Sept. 22, 1963 - Feb. 1, 1965

...

Senior Assistant (Neurology, Neuropathology)

Neurological Clinic of Medical Academy in

Warsaw - Poland, Dr. I. Hausman.

Sept. 1, 1961 - Sept. 21, 1963

...

Junior Assistant in Neurology,

Neurological Clinic of Medical Academy

in Warsaw-Poland, Dr. I. Hausman,

Sept. 1, 1959 - Sept. 1, 1961

...

BIOGRAPHICAL SKETCH

H.M.Sobkowicz

Education:

Girls State Lyceum, Gliwice- Poland

Baccalaureat

1943 - 1949

Medical Academy in Warsaw - Poland

Medical Doctor

1949 - 1954

1st Internal Medical Clinic of

Medical Academy in Warsaw - Poland

Intern

1954 - 1955

Neurological Clinic Of Medical

Academy in Warsaw - Poland

Resident

1955 - 1959

Board in Neurology : Sept. 29, 1959

Medical Academy of Warsaw - Poland

Equivalent of Ph.D. in Medical Science: 1962

Medical Academy of Warsaw - Poland; Thesis

from the Department of Neurology (Dr.I.Hausman)

" The Neurological Syndromes Due To Lead Poisoning"

Personal Publications (related to lead poisoning):

1.- H.M.Sobkowicz. The Neurological Syndromes Due To Lead Poisoning.

These de Doctorat en Medicine, Medical Academy of
Warsaw - 1962. (in Polish)

BIOGRAPHICAL SKETCH

- 3 -

H.M.Sobkowicz

- 2.- Z.Palkowska, H.M.Sobkowicz, J.Tur. Chronic Lead Poisoning with
Ophthalmic and Central Nervous System Damage.
Arch.Malad.Prof., 1961,22(12):759-761. (in French)
Pol.Tyg.Lek. 1964,19(1): 12-15 (in Polish)
- 3.- B.Adamowicz, T.Dorywalski, J.Kus, H.Sobkowicz. Changes in the Labyrin-
thine - Vestibular System in Chronic Lead Poisoning.
VI Nat.Conf.Ind.Med., Gdansk - 1962 (in Polish)
- 4.- I. Hausmanowa-Petrusewicz, B.Emeryk, H.Sobkowicz, B.Wasowicz, J.Tur.
EMG in Chronic Lead Poisoning. (in Polish)
Pol.Tyg.Lek. 1962,17:36.
- 5.- H.M.Sobkowicz, M.R.Murray, L.J.Goldwater, E.R.Peterson, M.B.Bornstein.
Influence of Lead Acetate On Development and
Maintenance of Nervous Tissue in Culture.
Manuscript in preparation.
- 6.- H.M.Sobkowicz, E.R.Peterson, M.R.Murray, L.J.Goldwater.
Absorption of Lead Acetate by Nervous Tissue in Cultur
Manuscript in preparation.
- 7.- H.M.Sobkowicz, M.R.Murray, L.J.Goldwater, E.R.Peterson, M.B.Bornstein.
Influence of Serum from Laboratory Animals with
Lead Poisoning on Nervous Tissue in Culture.
Manuscript in preparation.

BIOGRAPHICAL SKETCH

H. A. Kubinski

PREVIOUS WORK AND INVESTIGATOR'S CAREER

<u>Name</u>	<u>Date of Birth</u>	<u>Marital Status</u>
H. A. Kubinski	January 15, 1933	Married, 2 children
<u>Place of Birth</u>	<u>Sex</u>	
Warsaw, Poland	Male	
<u>Education</u>	<u>Degree</u>	<u>Year Conferred</u>
Lyceum, Pruszkow/Warsaw, Poland	BS	1950
University of Warsaw Medical School	MD	1955

Major Research Interest

Molecular biology, neurochemistry, virology

Research And Professional Experience

1965 - Department of Surgery, Division of Neurosurgery, University of Wisconsin Medical School, Assistant Professor.

From 1954-1955 - research assistant at Medical School Institute of Microbiology, Warsaw, Poland. 1956-1960 - research associate at the same institute.

Research: virus-cell interaction, adsorption and penetration of viruses.

1960-1964 - research associate at Stiftung zur Erforschung der spinalen Kinderlähmung und der Multiplen Sklerose (presently: Heinrich-Pette-Institute at University of Hamburg) Hamburg, Germany. Research on: nucleic acid metabolism in normal, neoplastic and virus-infected animal cells. Chemistry of virus nucleic acids.

1964-1965 - McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin, project associate.

Research on: interaction of nucleic acids.

The studies related to the proposed program have been pursued since 1960. They included studies on:

- (a) RNA and DNA metabolism in normal and neoplastic animal cells in vivo and in vitro as well as similar studies on the metabolism of nucleic acids in bacteria (ref. 5, 6, 9, 11, 12, 14 and 16).
- (b) Influence of various factors (virus infection, treatment with antibodies, drugs and irradiation) on the synthesis of RNA in animal cells (ref. 1, 2, 3, 5, 7, 8, 10 and 15).
- (c) Binding of polynucleotides on ribosomal RNA; conditions of such binding and detectability of complexes (ref. 12 and 18).

BIOGRAPHICAL SKETCH

-2-

H. A. Kubinski

(d) Separation of DNA strands and reactions between DNA and synthetic polyribonucleotides and between DNA and ribosomal ribonucleic acid (ref. 12, 17, 19 and 20).

(e) Methodology of extraction, separation and physico-chemical analysis of cellular nucleic acids (ref. 2, 4, 5, 7, 12, 13 and 17).

REFERENCES

- (1) H. Kubinski and G. Koch: On the Separation of Poliovirus RNA from Cellular Ribonucleic Acid. *Virology* **17**, 220, 1962.
- (2) H. Kubinski, G. Koch and O. Drees: Fractionation of Ribonucleic Acid from Poliovirus-Infected Cells by Column Chromatography. *Biochim. Biophys. Acta* **51**, 332, 1962.
- (3) H. Kubinski: Chromatography of RNA from Antibody-plus-complement treated Amnion Cells. Symposium on Experimental Contributions to the Pathogenesis of the Demyelinating Encephalomyelitides. Hamburg, August 2-4, 1962, in *Z. Immunitäts. Allergieforschung*, **126**, 184, 1964.
- (4) G. Koch and H. Kubinski: Column Chromatography Separation of Infective and Non-Infective Nucleic Acids from Poliovirus. *Z. Naturf.* **17b**, 655, 1962 (in German).
- (5) H. Kubinski and G. Koch: Instability of an RNA-fraction from Poliovirus infected Cells. *J. Mol. Biol.* **6**, 102, 1963.
- (6) H. Kubinski, G. Koch and B. Hieronymi: Influence of Nutritional Conditions on the Cellular RNA Metabolism *in vivo* and *in vitro*. *Experientia (Basel)* **19**, 311, 1963.
- (7) H. Kubinski: Effect of Ultra-violet Irradiation of Ribonucleic Acid on its Chromatographic Behaviour. *Nature (London)* **200**, 595, 1963.
- (8) G. Koch and H. Kubinski: Nucleotide Composition of RNA Synthesized in Human Cells Infected with Poliovirus. Biochemical Symposium Strasbourg, August 19-21, 1963; in *Bull. Soc. Chim. Biol.* **46**, 193, 1964 (in French).
- (9) H. Kubinski and G. Koch: On the Synthesis of RNA in Animal Cells under Suboptimal Conditions. *ibid.*, **46**, 194, 1964 (in French).
- (10) G. Koch and H. Kubinski: RNA with a High Cytosine and a Low Guanine Content in Poliovirus Infected Cells. *Biochim. Biophys. Res. Commun.* **15**, 308, 1964.

BIOGRAPHICAL SKETCH

-3-

H. A. Kubinski

- (11) G. Koch and H. Kubinski: Synthesis of High Molecular Weight RNA in Mammalian Cells. Vith Int. Congress of Biochemistry, New York, N.Y. July 26 - Aug. 1, 1964. Abstracts Vol. 1, p. 66.
- (12) Z. Opere-Kubinska, H. Kubinski and W. Szybelski: Interaction between Denatured DNA, polynucleotides and Ribosomal RNA: Attempts at Preparative Separation of the Complementary DNA Strands. Proc. Natl. Acad. Sci. U.S., 52, 923, 1964.
- (13) G. Koch and H. Kubinski: On the Preparation and Fractionation of High Molecular Weight RNA. Z. Naturf. 19b, 683, 1964 (in German).
- (14) G. Koch, H. Kubinski and G. Trams: Synthesis and Turnover of Ribonucleic Acids in Mammalian Cells in Suspension Culture. I. Influence of Metabolic State. Z. Naturf., 21b, 261, 1966 (in German).
- (15) G. Koch, G. Trams and H. Kubinski: Synthesis and Turn-over of Ribonucleic Acids in Mammalian Cells in Suspension Culture. II. Influence of Actinomycin. Z. Naturf. 21b, 967, 1966 (in German).
- (16) H. Kubinski and G. Koch: Regulation of the Synthesis of Ribonucleic Acids in Animal Cells. Biochem. Biophys. Res. Commun., 22, 346, 1966 and I. E. G. #7, Memo #215.
- (17) H. Kubinski, Z. Opere-Kubinska and W. Szybelski: Patterns of Interaction between Polynucleotides and Individual DNA Strands Derived from Several Vertebrates, Bacteria and Bacteriophages. J. Mol. Biol., 20, 313, 1966 and I. E. G. #7, Memo #347.
- (18) H. Kubinski, Z. Opere-Kubinska and W. Szybelski: Binding of Fast-Labeled RNA to Ribosomal RNA. Manuscript in preparation.
- (19) W. Szybelski, H. Kubinski and P. Sheldrick: Pyrimidine Clusters on the Transcribing Strand of DNA and their Possible Role in the Initiation of RNA Synthesis. Cold Spring Harbor Symposium on Quantitative Biology, 31, 1966 (in press).
- (20) H. Kubinski and J. A. Rose: Presence of Sequences Containing Repeating Base-Pairs in DNA from Some Oncogenic and Non-Oncogenic Animal Viruses, Proc. Natl. Acad. Sci. U.S., (in press).

BIOGRAPHICAL SKETCH

R. Monzain

Rachel Monzain, B.A., R.M.; Born: 12,4,1928, in Paris, France.

Project Assistant of Tissue Culture Laboratory of the Department of
Neurology, Chairman: Francis M. Forster, M.D.

University of Wisconsin Medical School.

August 1, 1967 - to date

Professional experience:

Laboratory for Cell Physiology,

Columbia University, Dr. M.R. Murray.

Research Worker

1966

Laboratory of Cellular Neurophysiology

Mt. Sinai Hospital, N.Y.C., Dr. M.B. Bornstein.

Research Assistant

1959 - 1966

Hopitaux de Paris, Bretonneau, Paris, France.

Dr. Dreyfus-Brisac.

EDS Assistant

1956 - 1959

Hopitaux de Paris, Boucicault Assistance

Publique, Dr. Pouthuill.

X-Ray Technician

1954 - 1955

Hopitaux de Paris, Cochin Assistance Publique,

Dr. J. Quenu.

Registered Nurse

1951 - 1953

Education:

College Sophie Germain, Paris, France.

Baccalaureat

1948

BIOGRAPHICAL SKETCH

- 2 -

R. Monzain

Ecole De la Salpetriere
School of Nursing ,Paris,France.

Registered Nurse

1951

Ecole de la Salpetriere:

X-Ray Technical School

1953

ECG Technical School

1955

Tissue Culture of The Nervous System.

Laboratory for Cell Physiology.

Dr. Margaret R. Murray

Columbia University

1959
