(Leave Blank)	Dr. Stanley Cohen
Received Date 3-2-59	Department of
Council Assigned June 1959	
Action	

HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF HEALTH

Mail Completed Application to:
Division of Research Grants
National Institutes of Health
Bethesda 14, Md.

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RG-6638
C.B. (1)
Formerly

APPLICATION FOR RESEARCH GRANT

Date February 6, 1959

Application is hereby made for a grant in the amount of $ 9,450. (not cents) for the period from September (month) 1 (day) 1959 (year) through August (month) 31 (day) ~~1964~~ 1960 (year) (RG), inclusive

for the purpose of conducting a research project entitled (Limit to 53 typewriter spaces).

Control mechanisms during embryonic development.

Check One:
- [x] NEW PROJECT
- [ ] RENEWAL OF PHS GRANT NO.
- [ ] SUPPLEMENT TO PHS GRANT NO.
- [ ] REVISION OF PHS APPLICATION NO.

Principal Investigator	Co-Principal Investigator, if any:
Name Stanley (First) Cohen (Middle) (Last)	Name (First) (Middle) (Last)
Title Assistant Professor	Title
Dept. Biochemistry	Dept.
School Medicine	School
University or Institution Vanderbilt University	University or Institution
Street Address	Street Address
City and State Nashville, Tennessee	City and State
Name, Title and Address of Financial Officer: Overton Williams, Bursar, Vanderbilt University, Nashville, Tennessee (RG)	Check to Be Drawn as Follows:

AGREEMENT

It is understood and agreed by the applicant: (1) That funds granted as a result of this request are to be expended for the purposes set forth herein; (2) that the grant may be revoked in whole or part at any time by the Surgeon General of the Public Health Service, provided that a revocation shall not include any amount obligated previous to the effective date of the revocation if such obligations were made solely for the purposes set forth in this application; (3) that all reports of original investigations supported by any grant made as a result of this request shall acknowledge such support; (4) that, if any invention arises or is developed in the course of the work aided by any grant received as a result of this application, the applicant institution will either (a) refer to the Surgeon General for determination, or (b) determine in accordance with its own policies, as formally stipulated in a separate supplementary agreement entered into between the Surgeon General and the grantee institution, whether patent protection on such invention shall be sought and how the rights in the invention, including rights under any patent issued thereon, shall be disposed of and administered, in order to protect the public interest.

NAME OF INSTITUTION: Vanderbilt University (RG)

ADDRESS:

CITY AND STATE:

NAME AND TITLE OF OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION (Please Type): John W. Patterson, Dean, School of Med.(RG)

PERSONAL SIGNATURE (This agreement must carry the actual signature of the official whose name appears on the line above): John W. Patterson (use ink)

Ber

PRIVILEGED COMMUNICATION

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Rev. 9-57

Form Approved
Budget Bureau No. 68-R249.7

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PROPOSED BUDGET for the period shown on page 1

NOTE: Under column entitled "OTHER" indicate funds presently available or anticipated from other sources, including those from own institution.	PERCENT OF TIME TO BE SPENT ON THIS PROJECT	BUDGET: REQUESTED FROM PHS (Omit Cents)	BUDGET: OTHER
PERSONNEL: Itemize All Positions, Indicating Type, Percent of Time To Be Spent On This Project and Names of Professional Personnel Selected.		$	$
Stanley Cohen, Senior Research Fellow	80		12,582.
Research Assistant (B.S. or M.S. level)	100	3000.	
Soc. Security, insurance		117.	
PERMANENT EQUIPMENT (See instructions reference itemization of equipment)		$	$
1 Binocular microscope		450.	
1 Beckman spectrophotometer with UV attachment		800.	1700.
1 Refrigerator		250.	
1 Low-temperature incubator		450.	
1 Incubator-shaker		650.	
CONSUMABLE SUPPLIES (Itemize)		$	$
General glassware		800.	
Chemicals		600.	
Animals		300.	
Microsyringes and pipettes		300.	
TRAVEL (State Purpose)		$	$
To attend scientific meetings and to consult with other workers when necessary		400.	
OTHER EXPENSE (Itemize)		$	$
Special Bibliographic Service		100.	

NOTE: The administrative official signing this application may add an amount for indirect costs.

IMPORTANT

Review detailed instructions before computing indirect cost allowance.

SUBTOTAL (DIRECT COSTS)	$ 8217.
INDIRECT COSTS	1233.
PHS PARTICIPATION ADJUST TO LOW DOLLAR	$
TOTAL BUDGET (OMIT CENTS)	9450. $

ESTIMATE OF FUTURE YEARS REQUESTED FROM PUBLIC HEALTH SERVICE

ADD'L YEARS	PERSONNEL	EQUIPMENT	SUPPLIES	TRAVEL	OTHER	SUBTOTAL (DIRECT COSTS)	INDIRECT COST ALLOWANCE	TOTAL
1st	$ 3737.	$ 2000	$ 2000	$ 400	$ 100	$ 8237	$ 1235	$ 9472
2nd	3737	1000	2000	400	100	7237	1085	8322
3rd	4149	1000	2000	400	100	7649	1147	8796
4th	4149	1000	2000	400	100	7649	1147	8796

If additional years requested are not contemplated enter "NONE" under total for first additional year.

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PUBLIC HEALTH SERVICE SUPPORT: Show current Public Health Service research grants and pending applications submitted to PHS.

GRANT NUMBER	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
ACTIVE SF-292	Senior Research Fellowship	12,582.- 14,892.	5 years
PENDING			

ALL OTHER SUPPORT: Excluding Public Health Service, but including that from own institution, list all other sources of research support and other pending applications. Use Continuation page if necessary.

SOURCE	TITLE OF PROJECT	AMOUNT	PERIOD OF SUPPORT
CURRENT			
PENDING			

RESEARCH PLAN AND SUPPORTING DATA

On the continuation pages provided give details of the proposed plan and other necessary data in accordance with the outline below. Number each page, the first continuation page being page 4. Additional continuation pages, if needed, may be requested from the Division of Research Grants. See detailed instructions before preparing this portion of the application.

1. RESEARCH PLAN
   - A. Specific Aims—Provide a concise statement of the aims of the proposed work.
   - B. Method of Procedure—Give details of your research plan. For each specific aim mentioned in "A" show how your plan is expected to fulfill the aim.
   - C. Significance of this Research—Explain why the results of the proposed work may be important.
   - D. Facilities Available—Describe the general facilities at your disposal. List the *major* items of permanent equipment.
2. PREVIOUS WORK DONE ON THIS PROJECT
   Describe briefly any work you have done to date that is particularly pertinent.
3. PERSONAL PUBLICATIONS
   Cite your most important publications on this or closely related work. List no more than five.
4. RESULTS OBTAINED BY OTHERS
   Summarize pertinent results to date obtained by others on this problem, citing publications deemed pertinent. Select no more than five.
5. BIOGRAPHICAL SKETCHES
   Provide brief sketches for *All* professional personnel selected who are to be actively engaged in this project.

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1. Research Plan

a) Specific Aims. Three general classes of hypotheses have been proposed in attempts to give a theoretical basis for the many observations on the differentiation of the fertilized egg into the tissues of the adult organism. Briefly, these involve a) the alteration in some way of the integrity of the genotype during differentiation, b) the existence of cytoplasmic self-reproducing units and finally c) the phenotypic changes are ascribed to the establishment in each of the various types of differentiated cells of one set of possible mutually exclusive metabolic steady states.

The major aim of the proposed researches will be obtain, in embryonic cells, experimental evidence with regard to some of these postulated control mechanisms of differentiation.

Biochemical evidence for the functional alteration in the DNA of differentiating cells must await a method for the detection of such changes. Among microorganisms the phenomena of transformation and transduction provide such a method. An attempt will be made to devise a similar system employing the undifferentiated cells of the early amphibian embryo as receptor cells.

The possibility that induced enzyme formation is one of the mechanisms by which a particular metabolic steady state is obtained in differentiated cells has been discussed for many years. Recently, such a steady state phenomenon, resembling in some ways differentiation in higher organisms, has been described by Melvin Cohn for the β-galactosidase system in E. coli. The apparent formation of an adaptive enzyme in frog embryonic cells (tryptophan peroxidase) has also recently been reported (Stearns and Kostellow). We propose to examine dissociated amphibian embryonic cells with respect to their abilities to form induced enzymes and to compare the results with the work which has been done using bacterial systems.

b) Method of Procedure.

1. Genetic integrity of the genotype during differentiation. The genetic integrity of the genotype has been assumed by most modern biologists until the question was reopened by the nuclear transplantation studies of Briggs and King with amphibian eggs. We also propose to use amphibian eggs in our studies. The major problem is to devise a method for the detection of such changes in the DNA, if they do indeed exist. The rational of our experiment is as follows: It is known that differentiated cells contain a variety of enzymes which are not detectable in the egg. For example, the adult frog liver contains histidase which is absent in the egg. DNA will be prepared from the liver of the adult frog (by procedures which have been used for the preparation of biologically active transforming factor and TMV nucleic acid) and measured amounts will be micro-injected into a fertilized frog egg. After suitable periods of incubation, the eggs will be examined for the presence of the specific adult enzymes. Similar kinds of experiments may be performed using RNA or microsomes. One major advantage of this system is that the eggs are sufficiently large to permit the injection of measurable quantities of material, thus avoiding difficulties arising from permeability barriers. Frog eggs are available throughout most of the year and microinjection procedures have been described in the literature and found to be workable in our hands.

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2. Induced enzyme synthesis during embryonic development. Artifically fertilized frog eggs will be allowed to develop to the desired stage and cell suspensions will be prepared with the aid of trypsin. Preliminary experiments in this laboratory have shown that such preparations display a marked increase in histidase activity after incubation with histidine. Similar results with tryptophan peroxidase have been reported by Stearns and Kostellow. To my knowledge the quantitative response of these embryonic cells with respect to their inductive capacity is much greater than has been reported in similar attempts using adult cells. Thus, in essence, we will apply the standard procedures of enzymology and bacterial enzyme induction to these cells, to elucidate the biochemical properties of the system, including such parameters as the specificity and kinetics of induction, the properties of the enzyme induced, the stability of the enzymatic activity during subsequent cell multiplication, and whether the observed activity represents a de novo synthesis of protein or the activation of the pre-existing enzyme.

c) Significance of this Research.

If it can be shown that the DNA of adult tissue can alter the metabolic pattern in the developing egg, it would provide suggestive evidence for the differentiation of nucleic acid during development and for the existence of a transformation phenomenon in higher organisms. Considerable further study would be required to establish this, and provide evidence for the proportion of the cells thus affected, and the stability of the changes induced. Although a negative result would not be very meaningful, the importance of a positive result would warrant such experimentation.

With respect to induced enzyme formation during embryonic development almost all of the discussions in the literature have been based on extrapolations from data obtained with microorganisms. Further progress must await the accumulation of information using embryonic systems.

2. Previous work done on this project.

Although not directly related to this project I have previously published a report on $C^{14}O_2$ fixation during early embryonic development of the frog embryo, in which the distribution of the $C^{14}O_2$ into the various compounds of unfertilized eggs, blastula, gastrula, and neurula were examined.

Preliminary unpublished experiments indicated that cell suspensions of early embryos could be induced to form histidase in the presence of histidine in the medium.

3. Personal publications.

1. Cohen, S. 1954 The metabolism of $C^{14}O_2$ during amphibian development. J. Biol. Chem., 211: 337-354.

2. Cohen, S. 1958 A nerve growth-promoting protein. p.665. Symp. on the Chemical Basis of Development. Johns Hopkins Press.

3. Cohen, S. and R. Levi-Montalcini. 1957 Purification and properties of a nerve growth-promoting factor isolated from mouse sarcoma 180. Can. Res.,17:15-20.

4. Cohen, S. and R. Levi-Montalcini. 1956 A nerve growth-stimulating factor isolated from snake venom. Proc. Nat. Acad. Sci., 42: 571-574.

5. Cohen, S., R. G. Frazier and H. H. Gordon. 1953 Metabolism of creatine and guanidoacetic acid in premature and full-term infants. Am. J. Diseases of Children. 86: 752-766.

4. Results obtained by others.

The nuclear transplantation studies of Briggs and King (1) show that endoderm nuclei of frog embryos show stabilized changes in their capacity to promote differentiation when transplanted into enucleated eggs. The component of the nucleus which changes is unknown.

Puck (2) on the basis of the morphological stability of mammalian cells plated as single isolates in tissue cultures has concluded that "the process of mammalian differentiation involves true changes in the genetic constitution of the daughter cells".

A model system for the stabilization of an altered phenotype has been demonstrated by Melvin Cohn (3) using the β-galactosidase system of E. coli. Pre-induced cells, when placed in a medium containing a low concentration of inducer will continue to synthesize enzyme whereas non-induced cells in the same medium will not.

Stearns and Kostellow (4) have shown that when dissociated cells of frog gastrula are incubated in the presence of tryptophan a marked increase in tryptophan oxidase activity occurs. Experiments by Gorden and Roder (5) indicate that chick embryonic cells are also responsive to substrate induction.

1. King, T. J. and Briggs, R., 1956 in Symp. on Quant. Biology, XXI, 271.
2. Puck, T. T. 1957 in Rhythmic and Synthetic Processes in Growth, 3, Princeton Univ. Press.
3. Cohn, M., 1958 in The Chemical Basis of Development, 458, Johns Hopkins Press.
4. Stearns, R. N. and Kostellow, A. B. 1958 in The Chemical Basis of Development, 448, Johns Hopkins Press.
5. Gorden, M. W. and Roder, J., 1953 J. Biol. Chem. 200: 859.

5. Biographical Sketch of Principal Investigator.

Stanley Cohen. Born: 1922. Brooklyn New York; A. B. 1943 Brooklyn College; M. A. (Zoology) 1945, Oberlin College. Ph. D. (Biochemistry, H. B. Lewis) 1948, University of Michigan; Instructor in Pediatr. Research, Univ. of Colorado 1948-52; Research Fellow, Amer. Cancer Society, Dept. of Radiochemistry (Dr. M. Kamen), Washington Univ., Medical School, 1952-53; Research Associate-Associate Professor, Department of Zoology, Washington University, 1953-59. Senior Research Fellow, Vanderbilt University, 1959--.

6. Justification of Specific Budgetary Requests.

The Beckman spectrophotometer will be the main analytical instrument used in these studies. Although other instruments are in the Department, they are in full use on other projects. The low-temperature incubator is required for the maintenance of frog embryos.
The request for $2000 for the second year is for partial cost of a refrigerated centrifuge. One is now available in the Department but by next year it is expected that the number of workers using it will increase and necessitate a second instrument.