GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 7/6/79

Project Title: Ontogenetic Regulation of Brain Monoamine Oxidase

Project No: G-32-659 Green Card

Project Director: Dr. James A. Diez

Sponsor: DHEW/PHS/NIH - National Institute of Mental Health

Agreement Period: From 7/1/79 Until 6/30/80 (01 year)

Type Agreement: Grant No. 1 R03 MH33393-01

Amount: $7,432 New PHS Funds (G-32-659)
391 GIT Contribution (G-32-328)
$7,823 Total

Reports Required: Annual Progress Report with Continuation Applications
Terminal Progress Report upon Grant expiration

Sponsor Contact Person (s):

Technical Matters

Contractual Matters
(thru OCA)

PHS Grants Management Official
Bruce L. Ringler
Chief, Grants and Contracts
Management Branch
National Institute of Mental Health
DHEW/PHS/NIH
Bethesda, MD 20014

Defense Priority Rating: none

Assigned to: Biology (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director–EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)
Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION SHEET

Date 6/4/82

Project Title: Ontogenetic Regulation of Brain Monoamine Oxidase

Project No: G-32-659

Project Director: Dr. James A. Diez

Sponsor: DHEW/PHS/NIH - National Inst. of Mental Health

Effective Termination Date: 6/30/80

Clearance of Accounting Charges: 6/30/80

Grant/Contract Closeout Actions Remaining:

NONE

- ☐ Final Invoice and Closing Documents
- ☐ Final Fiscal Report
- ☐ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

Assigned to: Applied Biology (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator	Research Security Services	EES Public Relations (2)
Research Property Management	~~Reports Coordinator~~ (OCA)	Computer Input
Accounting	Legal Services (OCA)	Project File
Procurement/EES Supply Services	Library	Other _____

FORM OCA 10:781

FORM APPROVED
OMB NO. 68-R1452

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTE OF MENTAL HEALTH

FINAL REPORT GUIDELINES

Month | Year
(01) (02) (03) (04)

INSTRUCTIONS

PHS policy requires that grantees submit a "terminal progress report" (final report) within 90 days after completion of the grant.

Please complete this series of items *as this final report.* The report will be filed with your applications, reports and other grant business in NIMH's central files. It will be read by staff in research program areas, and may be read by other Institute staff concerned with program analysis, communication, evaluation and planning. The report will be used for information about your research, i.e., to describe and summarize the information *(procedural as well as substantive)* resulting from NIMH support, and to relate that information to mental health problems and research. Your report will often be used apart from your application; however, other documents, such as publications and applications, will be available from the project file if needed.

These guidelines have been designed with relatively small response spaces to encourage brevity. However, do not restrict your response if more space is needed: be complete, using additional labeled pages inserted where necessary *(sample page included).* Extensive descriptions and discussions, if desired, should be made *in addition to* your summary response to the item, and should be placed as appendices. Discussions of issues not covered by these guidelines are also welcome as appendices. Use clear, concise language, avoiding highly technical language *where practicable (this will vary for different types of research);* appendices could be more technical than responses to the items.

All publications resulting from this project, and not previously submitted, should be submitted with this report *(or as soon as available);* see the section on Dissemination. Publications should *not* be used in lieu of responses to particular items.

Send copies of this report and all appendices as indicated below.

All Grants
Send 3 copies *

TO: Grants Closeout Unit
Grants Management Branch
Office of Program Support
National Institute of Mental Health
5600 Fishers Lane, Room 7C-18
Rockville, Maryland 20857

* *Send two copies only of any books included.*

FOR NIMH USE ONLY

Branch/Section: ___ ___ - ___

ADMINISTRATIVE DATA:

(NOTE: *If items 1-4 have changed, give the latest information)*

1. GRANT NUMBER

R	0	3	M	H	3	3	3	9	3
(05)	(06)	(07)	(08)	(09)	(10)	(11)	(12)	(13)	(14)

2. TITLE OF GRANT
Ontogenetic regulation of brain monoamine oxidase

3. NAME OF PRINCIPAL INVESTIGATOR
James A. Diez

4. SPONSORING INSTITUTION
Georgia Institute of Technology

SIGNATURE OF PRINCIPAL INVESTIGATOR

5. NAME AND POSITION OF PERSON WRITING THIS REPORT IF OTHER THAN ITEM 3

ADM 442
Rev. 8-80

CONTINUE ON REVERSE SIDE

AIMS OF THE PROJECT:

(PROBLEM STUDIED)

6. Describe briefly the *specific aims* of your project, indicating major changes in direction from the original aims:

The purpose of this project was to determine the feasibility of using primary mouse brain cell cultures as a model system for studying the genetic and endocrine control of monoamine oxidase (MAO) in developing brain. To evaluate "feasibility," the following specific aims were proposed:

"1. Identification and characterization of genetic variation in brain MAO.

"Genetic variation in MAO will be identified in screening experiments of several inbred mouse strains. Types A- and B- MAO will be assayed in brain homogenates from neonatal and adult mice....

"Genetic variation in MAO will be characterized by determining the kinetic constants Km and Vmax for each form of the enzyme and by an examination of the rates of change in enzyme activity during early postnatal development.

"2. Determination of the effects of hormone treatment on MAO during the early postnatal period.

"The following hormones will be administered to groups of mice for the first 3-7 days of life: estradiol, testosterone, corticosterone, and thyroxine. MAO-A and -B will then be assayed in brain preparations at days 8, 15, 30, and 60. If genetic variation in MAO was discovered (#1, above), the hormones will be administered to genotypes showing the greatest differences....

"3. Comparison of results obtained from fresh tissue with analogous experiments utilizing cultured brain cells.

"Primary brain cell cultures will be started using the mouse genotypes found to show the greatest differences in vivo. MAO activity in the cultured cells wil be characterized in order to determine whether the genotype-dependent variation is maintained or expressed in vitro.

"Hormone treatments which were found to be effective in vivo will also be tested in the cell cultures."

7. Were the aims pursued as *originally formulated?*

1 ☒ Yes
2 ☐ No
(15)

TYPE OF RESEARCH:

8. In general, how would you *characterize* your research?
(Rank any multiple answers, using "1" as most appropriate)

(16) ☒ Hypothesis development
(17) ☐ Hypothesis testing
(18) ☐ Development or refinement of methodology
(19) ☐ Gathering of data; e.g., surveys
(20) ☐ Other *(Specify):*

CONDUCT OF RESEARCH:

9. Describe the *methodology* used in your research, including characteristics of any sample used:

MAO activity was assayed in homogenates prepared from fresh mouse brain and from brain cells which had been cultured from fetal mice for 1 to 3 weeks in vitro.

All of the mice used were bred in my colony from commercially available strains. Adult mice were sacrificed by cervical dislocation; decapitation was used for fetal and neonatal mice. The gestational age of fetuses used for brain cell cultures was determined by recording dates of occurrence of vaginal plugs; daily birth records were used for determining postnatal age.

For brain cell cultures, brains from 16-18 day fetuses were dissociated by trypsinization; the cells were grown in monolayer cultures in plastic dishes in Dulbecco's modified Eagle's medium.

Two forms of MAO were assayed: ^{14}C-5-hydroxytryptamine was used as the substrate for MAO-A; ^{14}C-B-phenylethylamine was used to measure MAO-B activity. The assays were adapted from published methods and were validated for appropriate kinetics and specificity under the conditions routinely used in these experiments.

10. Did you have significant *technical methodological* difficulties?
(Examples: necessary measurement tools undeveloped; unexpected inadequate data base)
If yes, describe, and explain how you dealt with them.

1 ☐ Yes
2 ☒ No (21)

11. Did you have significant *practical operational* difficulties?
(Examples: trouble with equipment; loss of sample or data; difficulties with cooperating units)
If yes, describe, and explain how you dealt with them.

1 ☐ Yes
2 ☒ No (22)

12. Describe (a) your *conclusions* or *results* as they relate to your specific aims *(please include negative results)*, and (b) their *significance* in relation to the field. Avoid highly technical language where practicable.

RESULTS:

a.) Some genetic differences in brain MAO were found in adult mice. The largest difference was in MAO-B, where the maximum activity (Vmax) was 72% higher in C57BL/10 mice than in Swiss albinos (an outbred strain) and 62% higher than in BALBc's (an inbred strain). The Km (a measure inversely related to the affinity of the enzyme for substrate) was also higher in the C57BL/10 mice (by 36% and 22%, repectively). Inbred DBA/1 mice had intermediate Km and Vmax values. Mixing brain homogenates from C57 and BALB mice before assay did not yield evidenc for enzyme activators or inhibitors in these strains.

For MAO-A, the Vmax in DBA/1 mice was 18% higher than in Swiss mice, but the Km's were hardly different (7-8% higher in DBA).

The genetic differences in MAO were found throughout postnatal development, but the pattern of change was different for MAO-A and -B. For type A, the Km showed a 30% increase between days 4 and 9 of life, with little or no change after that. The Vmax increased by 75% fromd days 3 to 9, and decreased after weaning to 67% of the highest values by adulthood. For MAO-B, the Km showed no change between day 3 and adulthood, but the Vmax increased 10-fold, with no sharp inflection point.

When the developmental-genetic differences were studied in brain cell cultures strain differences in the Km and Vmax for MAO-A were maintained for 3 weeks, but for MAO-B in the same cultures, the differences disappeared after 1 week in vitro. These results suggest that the difference in MAO-A is inherent in the brain cells, but the expression of the difference in MAO-B depends upon the environment in which the cells develop.

Attempts to find hormonal treatments which affected the developmental changes in MAO in vivo and in vitro were unsuccessful.

b.)Since some drugs which affect MAO activity are effective in treating human behavioral disorders, it is possible that endogenous abnormalities in MAO are part of the causal mechanism of the behavioral disorder. It is therefore important to learn how this enzyme is regulated physiologically. The work supported by this grant has not made a major contribution to answering this question, but it has demonstrated the feasibility of an in vitro approach which may be useful in studying various wasy by which gene activity can differentially affect MAO-A and -B activity.

RESULTS
(Continued)

13. Did you have *other findings* not directly related to the specific aims *("serendipitous findings")?*

If yes, describe:

1 ☐ Yes
2 ☒ No (23)

14. How do the *overall results* of your project fit into these descriptions? *(If you had multiple expectations or hypotheses, base your response on the predominant trend of the results).*

☒ Confirming your hypotheses or expectations (24)

☐ Disproving your hypotheses or expectations (25)

☐ Inconclusive (26)

15. Did your research result in significant *methodological developments?*

If yes, describe:

1 ☐ Yes
2 ☒ No (27)

IMPLICATIONS:

16. How would you describe the *impact* of your project?
(Rank any multiple answers, using '1" as most appropriate)

(28) ☐ Opening up a new line of research
(29) ☒ Contributing to the knowledge base of the field
(30) ☐ Providing facts ready for application in a field
(31) ☐ Indicative of a "dead-end" line of pursuit

17. Do you have immediate plans for *further research* in this area? 1 ☒ Yes (32) 2 ☐ No
If yes, describe:

Although I have some other things which need more "immediate" attention, I do plan to do further research in this area within the next year.

The differential behavior of MAO-A and -B in brain cell cultures seems to be a good system in which to explore the ways the two forms of the enzyme are regulated. The observation that the genetic differences in MAO-A are maintained in culture, while those in MAO-B disappear, should lead to a better understanding of how gene activity in the central nervous system is regulated.

18. Beyond your own plans, what is your opinion of the future directions this research area should take?

In general, much more needs to be known about the genetic and humoral (e.g., endocrine) regulation of brain metabolism. This enzyme system may be a good one to study further becuase it is clinically relevant, relatively easy to study, and some of the physiologically important determinants have been identified.

19. Do you have *specific suggestions (experiments, cautions, etc.)* for other research in this area? 1 ☒ Yes (33) 2 ☐ No
If yes, describe:

Given the differential regulation of MAO-A and -B, it is now difficult to justify the assay of "MAO activity" with a non-specific substrate such as tryptamine, which is acted upon by both forms of the enzyme. In fact, some earlier work in which the sub-types were not differentially assayed, should probably be reevaluated.

IMPLICATIONS

(Continued)

20. Are you aware of *other researchers* using your techniques, or planning to replicate your study, or of some individual or organization continuing your work? *If yes, describe, and check the type of impact which best characterizes the impact of your research at this time.*

1 ☐ Yes
2 ☒ No (34)

☐ Specific utilization (35)

☐ General field impact (36)

DISSEMINATION:

21. As an appendix, list *all publications (and articles accepted for publication)* resulting from this project. Send any publications which have not already been submitted as *appendices,* with grant number indicated on each. *(See instructions, page 1, regarding submission of books)*

22. Do you have any plans for future publications, papers, and/or demonstrations dealing with the results of this project? If so, describe briefly. Send in any future publications based on this project as per instructions on page one.

1 ☒ Yes
2 ☐ No (37)

One paper is In Preparation.

APPENDICES:

See instructions, page 1, paragraph 3.