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GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 2/1/79

Project Title: Aging Effect of Radiation on a Dormant Nematode

Project No: G-32-650 Green card

Project Director: Dr. Edward K. Yeargers

Sponsor: DHEW/PHS/NIH - National Institute on Aging

Agreement Period: From 1/1/79 Until 12/31/79 (01 Year)

Type Agreement: Grant No. 1 R01 AG01061-01

Amount:
$15,133 New PHS Funds (G-32-650)
797 GIT Contribution (G-32-323)
$15,930 Total

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

Sponsor Contact Person (s):

Technical Matters
Betty H. Pickett, Ph.D. (Dr. Murphy)
Assoc. Director for Extramural and
Collaborative Research Programs
National Institute on Aging
Bethesda, MD 20014

Contractual Matters
(thru OCA)
Ruth S. McClure
Grants Management Officer
National Institute on Aging
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

CA-3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 2/18/81

Project Title: Aging Effect of Radiation on a Dormant Nematode

Project No: G-32-650

Project Director: Dr. Edward K. Yeargers

Sponsor: DHEW/PHS/NIH - National Institute on Aging

Effective Termination Date: 12/31/79

Clearance of Accounting Charges: -----

Grant/Contract Closeout Actions Remaining:

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

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
Project File (OCA)
Project Code (GTRI)
Other OCA Research Property Coordinator
Suspense

CA–4 (1/79)

GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA 30332

OFFICE OF
THE
COMPTROLLER

May 28, 1980

Grants Management Officer
National Institute on Aging
DHEW/PHS/NIH
Bethesda, Maryland 20205

Dear Sir or Madam:

Enclosed is the Final Report of Research Grant Expenditures (form HEW-489) for Grant No. 1 R01 AG01061-01 covering the period January 1, 1979 to December 31, 1979.

If you have any questions or require additional information, please let us know.

Sincerely,

David V. Welch, Manager
Grants and Contracts Accounting

DVW/BITS/jb
Enclosure
cc: Dr. E. K. Yeargers
Dr. J. W. Crenshaw, Jr.
Mr. E. E. Renfro
Mr. O. H. Rodgers
File G-32-650

Department of Health, Education, and Welfare

NAME AND ADDRESS OF GRANTEE INSTITUTION	TRANSACTION NO.	Grant No.
Georgia Institute of Technology Atlanta, Georgia 30332	(08) R1AG01061A	1 R01 AG01061-01
	INSTITUTIONAL ID NO.	DATE OF THIS REPORTING PERIOD
	G-32-650	FROM 1/1/79 TO 12/31/79
		PROJECT PERIOD
		FROM 1/1/79 TO 12/31/79
		☒ CHECK IF FINAL REPORT

1. Expenditures of DHEW Funds for this Reporting Period

a. Personnel	$	h. Alterations and renovations	
b. Consultant services		i. Other	
c. Equipment			
d. Supplies		j. Total direct costs	$ 9,434.97
e. Travel, domestic		k. Indirect costs: Rate 76 % ☒ S&W ☐ TDC	
f. Travel, foreign		Base $ 7,472.00	$ 5,678.72
g. Patient care costs		l. TOTAL	$ 15,113.69

2. Expenditures from Prior Periods (previously reported)	-0-
3. Cumulative Expenditures	15,113.69
4. Total Amount Awarded — Cumulatively	15,133.00
5. Unexpended Balance (Item 4 less Item 3)	19.31
6. Unliquidated Obligations	-0-
7. Unobligated Balance (Item 5 less Item 6)	19.31
8.a. Cost Sharing Information — Grantee Contribution This Period	797.21
b. % of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 5.0
9.a. Interest/Income (enclose check)	
b. Other Refundable Income (enclose check)	

10. Remarks

I hereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in accordance with appropriate grant policies and for the purposes set forth in the application and award documents.

Dr. E. K. Yeargers Associate Professor — May 27, 1980 (Date)

SIGNATURE OF INSTITUTION OFFICER — 5/28/80 (DATE)

David V. Welch, Manager, Grants & Contracts Accounting
404/894/4624

REPORT OF RESEARCH GRANT EXPENDITURES

Edward K. Yeargers
435-52-8113

Final Report: Project # 1-R01-AG0 1061-01 RAD

Project Name: The work to be described here was performed between January 1, 1979 and December 31, 1979. The title of the project is "Aging Effect of γ-Radiation on a Dormant Nematode".

Personnel: The Principal Investigator was Dr. Edward K. Yeargers, Associate Professor of Biology, who served during the full grant period at 25% time. No other professional personnel were involved.

Summary: The purpose of this research was to investigate the life-shortening effect of an externally-applied insult to a nematode while this animal was in a state of slow metabolism - the dauer state. I found that 6×10^4 rad of γ-rays given during the "non-aging" state have a real, but small, effect on the lifespan of the worm - following resumption of the normal life cycle. The results are consistent with either of two explanations: a. Aging in this nematode is mainly controlled by an internal, pre-programmed mechanism which is largely unaffected by large doses of ionizing radiation. b. The nematode has a very efficient radiation-resistant repair mechanism for damage from ionizing radiation, even when in the dauer condition.

The Experimental System: Caenorhabditis elegans is a free-living, normally-hermaphroditic nematode. It has been used in studies on aging in a number of laboratories because of its ease of culture, small size (adults are about 1 mm long), short lifespan, specific aging symptoms, and simple body-organization (1). C. elegans undergoes four "obligate" larval stages (L1-L4) following hatching of the egg. At 20°C, the adult, reproductive stage occurs about three days after hatching. Four days later, egg-laying ceases and degenerative changes of physical, chemical, and morphological natures associated with aging become noticeable; death occurs about 10 days later, depending on the specific culture techniques used. Careful work in several laboratories has resulted in accurate data on the morphological and cellular changes which occur in C. elegans as it progresses through its life cycle (2-6). The various developmental changes between laying of the egg and the death of the adult occur at reasonably predictable times for given culture conditions.

If C. elegans larvae are starved early (starting at about 10 hours), they enter the "dauer" stage at the second molt, i.e., at the end of L2 (4,5). Compared to normal L3 worms, dauer larvae apparently are in a much-lowered metabolic state; resumption of feeding and a return to normal growth follow exposure to sufficiently high food concentration. It has been reported, however, that the post-dauer survival time of C. elegans is independent of the length of the dauer stage. Thus, C. elegans apparently may not age significantly during that period (6).

Report on the Work: Inasmuch as the post-dauer lifetime of C. elegans is independent of the time spent by the worm as a dauer larva, it can thus be inferred that dauer larvae do not age. This observation suggests strongly that external insults (e.g. cosmic radiation, air pollutants) may be far less important in aging in C. elegans than preprogrammed aging. I originally proposed that an external insult - in the form of large doses of γ-rays - would test the ability of C. elegans dauer larvae to resist or repair

radiation-induced damage. If the post-dauer-survival was minimally affected by the γ-rays, the primacy of internal, as opposed to external, factors in aging would be buttressed.

Newly formed dauers were isolated and suspended in a non-nutritive nematode buffer, called M9, at a concentration of about 100/ml, in a cotton-stoppered test tube (3). Gamma-ray doses of 0, 5, 10, 25, or 60 krad were administered by placement of the worms at different distances from a cobalt-60 radiation source. These doses were given continuously over 3, 6, 10 or 20 day periods. After irradiation the dauers were placed on agar plates which contained their normal bacterial food; reversion to obligate stages by the worms occurred within 12 hours. The obligate worms were checked daily until they died.

Results and Discussion: The results are given in Table 1. Ninety-five percent confidence intervals for the mean lifetime are given in order that individual mean lifetimes can be compared. However, a comparison of variation in all lifetimes with dose alone (independent of irradiation time) or of variation in all lifetimes with irradiation time alone (independent of dose) cannot be made accurately with such confidence intervals, inasmuch as such pooling of data leads to large errors. Thessse kinds of comparisons can be made satisfactorily by the method of analysis of variance, a procedure which was run on the data of Table 1. Analysis of variance shows that the differences in mean lifetimes with dose alone and irradiation time alone are significant at the 0.1% level. This indicates that the effects of dose and irradiation time on the post-dauer lifetimes are real although, from consideration of the overlap of the individual confidence intervals in Table 1, small. The major variation apparently is a post-dauer lifetime increase after 10 krad of radiation given over three to ten days.

These data indicate that, using post-dauer lifespan as an index, C. elegans dauer larvae are rather tolerant of ionizing radiation compared to most organisms. A number of insects and some microorganisms also can withstand several tens of kilorads before showing effects (7). Rather than being interested in mortality curves, my purpose was to apply to the "non-aging" nematode a stress which normally has the effect of shortening lifespan. With this in mind, it is clear that a considerable radiation stress has only a small effect on post-dauer survival time.

Two different explanations, taken separately or together, are possible: First, it may be that aging, as measured here, is rather strictly under internal control in the nematode - as long as the animal does not suffer gross mechanical injury. The nature of such an internal mechanism is unclear; it would have to be resistant to large doses of radiation. A small, non-protein hormone, for instance, might be appropriate. Its size would give a small probability of being "hit" in an inactivation event; proteins are inactivated, typically, by doses of the order of 10 krad. Second, the γ-radiation may well be causing damage which could affect aging, as measured here, but the damage is repaired. The evidence for repair of radiation damage is extensive (8). The usual interpretation of the dauer state, however, is that it is a metabolically-depressed state, O_2 consumption being only about 10% that of obligate stages for the same worm (9). Thus, the likelihood of a repair mechanism would be lessened inasmuch as it would surely require energy. On the other hand, the question of whether a "dormant" dauer larva actually has a metabolically depressed repair system has not ever been addressed, to my knowledge.

Literature Cited:

1. Zuckerman, B.M., in Spec. Rev. Exp. Aging Res., ed. Elias, M.F., p. 429 (1976).
2. Sulston, J.E., and H.R. Horvitz, Dev. Biol. 56 110 (1977).
3. Byerly, L., R.C. Cassada, and R.L. Russell, Dev. Biol. 51 23 (1976).
4. Hirsh, D., D. Oppenheim, and M. Klass, Dev. Biol. 49 200 (1976).
5. Cassada, R.C., and R.L. Russell, Dev. Biol. 46 326 (1975).
6. Klass, M. and D. Hirsh, Nature 260 523 (1976).
7. Bacq, F., and P. Alexander "Fundamentals of Radiobiology," Pergamon Press 1961 p. 301.
8. Alper, T., in "Sterilization of Medical Products by Ionizing Radiation", International Conference, Vienna 1977, Multisciences Publ. 1978, p. 9.
9. Dr. G. Anderson, Georgia Institute of Technology, Personal communication (1979).

Publication Submitted to the Journal of Nematology:

"Aging Effect of γ-Radiation on Dauer Larvae of Carnorhabditis elegans"

Table 1: Average post-dauer lifetimes ($\bar{\tau}$), 95% confidence intervals of the sample mean (CI_{95}), and sample sizes (n) for different irradiation times and doses.*

Irradiation time in days	Irradiation dose in krad				
	0	5	10	25	60
0	τ = 9.4 CI_{95} = ± 0.5 n = 61				
3	9.6 ±0.8 29	9.6 ±1.0 27	10.1 ±0.6 34	9.2 ±0.6 31	9.0 ±0.8 35
6	9.7 ±0.4 40	9.6 ±0.5 43	10.2 ±0.6 45	9.5 ±0.6 35	9.3 ±0.4 92
10	10.2 ±1.2 27	10.9 ±0.9 17	12.0 ±0.6 23	9.3 ±0.7 22	9.8 ±0.6 42
20	10.2 ±0.5 53	9.7 ±0.4 52	9.6 ±0.4 44	9.7 ±0.4 42	9.1 ±0.4 71

*Notes: a. The lifetime of each worm is accurate to one day. The extra decimal in the above data reflects the fact that, with large sample sizes, the average is more accurate than its components.

b. CI_{95} is that interval about the sample mean within which one can be 95% certain that the true mean lies. This provides a method for comparing lifetimes for different doses and/or irradiation times. As explained in

(Notes: continued on next page

(Notes: continuation)

the text, analysis of variance methods showed overall effects of dose alone and time alone which were both significant at the 0.1% level.