COO-3269-5

FINAL COMPREHENSIVE REPORT OF OVERALL ACTIVITIES OF AEC CONTRACT AT (30-1) 3269 FROM ITS INITIATION

This document is an addendum to the annual progress report. This addendum contains the following sections:

A. Main research accomplishments with special reference to original objectives.

B. Graduate students trained, degrees granted, post-doctoral tenures completed, medical school trainees, college and high school trainees.

C. Bibliography with titles of publications associated with this contract.

The title of the original research proposal funded under Contract 30-1-3359 was Specialized Responses to Ionizing Irradiation, Part I: Fibrinogen Deposition in vivo after X-Irradiation; Part II: Alteration of Taste Thresholds by Low Doses of Ionizing Radiation. Upon the completion of the fourth year of the contract, the principal investigator was advised to devote his full time research interests to the Alteration of Taste Thresholds portion of the original contract. Support was terminated at the end of the fifth year for the Fibrinogen Deposition in vivo after X-irradiation segment of the program. The last four years of the contract was devoted solely to further studies of changes in taste thresholds following low doses of X-irradiation. Considerable information has been gathered concerning fibrinogen deposition in vivo; these results will also be presented in this comprehensive report, and will follow description of our observations of changes in taste threshold by ionizing irradiation.

I. Alterations of Taste Thresholds by Low Doses of Ionizing Irradiation

A. Main Research Accomplishments

The original objectives of this portion of the research program were:

1. to determine the minimum level of radiation in rats to alter the taste threshold.
2. to determine the permanency of such alteration.
3. to determine the dose and time dependency of the alteration.

All of the original objectives have been accomplished.

DISCLAIMER

1. To Determine the Minimum Level of Radiation in Rats to Alter Taste Thresholds

We were able to demonstrate a ten-fold decrease in the taste acuity for saccharine (sweet), sodium chloride (salt), quinine hydrochloride (bitter) and hydrochloric acid (sour), in adult male Wistar rats following 50 R of 250 KVCP tongue X-irradiation. This was possible because our behavioral technique for determining taste thresholds using lateral hypothalamic stimulation for the reward stimulus was extremely sensitive. The pre-irradiation taste threshold values for saccharine, 0.001%; sodium chloride, 0.002%; hydrochloric acid, 0.001%; and quinine hydrochloride, 0.0001%; were three to six times lower than previously reported values. We have also demonstrated in our laboratory that a less severe effect on taste thresholds can be produced with exposure doses as low as 30 R. With exposures below this level we have not been able to demonstrate a statistically significant change in the taste threshold for the materials used. Refinement of our behavioral taste threshold determination technique, and an increase in the sample size, may permit validation of threshold alterations below 30 R.

2. To Determine the Permanency of Such Alteration

The ten-fold decrease in taste sensitivity following 50 R of X-irradiation returned to normal levels within 72 hours. The speed of onset of the alteration differed when different taste materials were being studied. The taste sensitivity for saccharine and quinine hydrochloride was seen within two hours following X-irradiation (the first post-irradiation taste threshold determination.) The thresholds for sodium chloride and hydrochloric acid showed a gradual decrease at six hours and did not reach a maximum until 24 hours following X-irradiation. All thresholds returned to normal within 72 hours independent of the type of material under investigation.

3. To Determine the Dose and Time Dependency of the Alteration

The decrease in taste sensitivity following X-irradiation has been observed with doses as low as 30 R. The same temporal relationships exist as seen following 50 R of exposure. There is a uniform decrease in the severity of the change and perhaps our technique of determining taste thresholds is presently not sufficiently sensitive to detect changes below the 30 R level. The changes seen at 30 R last for 72 hours, as they do following 50 R of exposure.

B. Supplemental objectives to the original objectives have been added yearly with each progress report and renewal request. These are as follows:

4. To observe changes in hypothalamic function following low doses of ionizing radiation manifested by alteration in:

   a. histology
   b. self-stimulation equilibrium rates

5. To develop a new sensitive behavioral technique for determination of taste thresholds.

6. To correlate taste sensitivity changes with alteration in taste bud morphology by:

   a. histologic examination of taste buds following tongue irradiation, through conventional light microscopy and electron microscopy
   b. incorporation rates of tritiated thymidine into taste bud cells

7. To determine whether olfaction significantly affects taste thresholds.

8. To determine the properties of the taste material that influences the radiation effect on taste.

9. To determine the effect of in utero X-irradiation on taste function in the adult rat.

10. To determine the effect of ingestion of heavy metals on taste acuity and the response of taste sensitivity to X-irradiation.

B. Supplemental Research Accomplishments

4. To Observe Changes in Hypothalamic Function Following Low Doses of Ionizing Radiation

Under our experimental conditions, constant stimulating potential (one volt rms), constant stimulating current (50 microamperes), and constant stimulus duration (0.3 seconds), there was no observable post-radiation difference in the rate of bar pressing in order to receive lateral hypothalamic stimulation. There was no statistically significant change in the rates of bar pressing in all animals

before and after irradiation at all doses tested (300 to 1800 R, head only irradiation). This was determined by comparing the slope of the fitted regression line with the null hypothesis, slope equals zero. The probability of "t" (single-tailed test) was greater than 0.10. There was a slight increase in daily performance deviation, as observed by other investigators. However, it was concluded that hypothalamic stimulation could be used as the reward mechanism in the taste threshold portion of the project.

5. To Develop a New Sensitive Behavioral Technique for Determination of Taste Thresholds

Taste thresholds in the rat have been determined utilizing various methods ranging from simple two-bottle preference tests to an involved conditioning protocol as described by Koh and Teitelbaum. These methods have drawbacks that limit their usefulness to specific experimental situations. It was necessary for us to devise a reliable method for determining taste thresholds in the rat for different taste solutions that 1) permitted rapid frequent determinations of taste thresholds, 2) maintained the animal's motivation to discriminate between test solutions throughout the entire threshold determination, and 3) did not require a tedious and prolonged training period. It was for these reasons that a conditioned-suppression behavioral technique was applied to our experimental situation. There was one important departure from the methodology previously reported using conditioned-suppression as an animal psychophysical technique. We were the first to use hypothalamic stimulation rather than food as the reinforcement stimulus. Lateral hypothalamic stimulation was chosen as the method of reward for two reasons: 1) it maintains the motivation without satiation and results in no post-reinforcement pause, as occurs with food reward. 2) The reinforcement is not a discernible taste substance (such as a food pellet) which would interfere with the measurement of an absolute taste threshold.

Hypothalamic stimulation was efficacious in producing stable rates of conditioned performance, and absolute threshold balues were obtained within three to four weeks following surgical implantation of the electrodes, and two to three weeks after beginning of training. After training had been completed, a threshold value for a particular material was obtained in 20 minutes, permitting application of this technique to our protocol where transient alterations in tast thresholds occured. Multiple determinations of the taste threshold of one animal were obtained during a 24 hour period since actual fluid consump-

tion was low (10 ml per determination), and satiation of the reward did not occur. And most important, the technique was sensitive with the determined threshold values: saccharin, 0.0010%; sodium chloride, 0.0020%; hydrochloric acid, 0.001%; and quinine hydrochloride, 0.0001%, which were three to six times lower than the previously reported behavioral values for these substances.

6. To Correlate Taste Sensitivity Changes with Alteration in Taste Bud Morphology

Changes in taste threshold were observed within two to six hours following irradiation to the tongue, and normal function returned by 72 hours. No appropriate morphological change following 50 R of x-ray exposure could be found in any of the tongues examined with conventional light microscopy during a three-day post-irradiation observational period.

There was no discernible difference in the rate of migration of tritium labelled cells into the taste buds before and after tongue irradiation. The rather slow appearance of tritiated thymidine in the taste bud by migration from the germinal centers was confirmed. An extrapolated life span of 10 days was estimated for a taste cell in the buds. An occasional taste bud had a labelled cell by 24 hours. The immediacy of the observed radiation effect, and the prolonged turnover time of taste bud cells, suggests that the site of low dose radiation interference with taste is independent of taste bud cell renewal kinetics.

7. To Determine whether Olfaction Significantly Affects Taste Thresholds

The absolute taste threshold values for saccharin, quinine, hydrochloric acid, and sodium chloride were determined in 32 adult Wistar rats, eight rats for each taste material, using the conditioned-suppression behavioral protocol. Lateral hypothalamic stimulation was used as the conditioning stimulus. Half the rats, four from each group, were rendered anosmic by olfactory bulb removal prior to electrode implantation. The efficacy of removal of the bulbs was proven prior to electrode implantation using a searching-for-food behavioral test described by Kahn and Stellar (1960). Gross inspection at autopsy and histological verification of the completeness of removal of the olfactory bulb was performed on each animal after completion of their threshold determination.

No differences in the pre-irradiation taste thresholds or in the radiation response could be observed whether the animals were normal or anosmic. Anosmia had no effect on taste perception at threshold levels.

8. To Determine the Properties of the Taste Material that Influence the Radiation Effects on Taste.

The taste threshold value, before and after 50 X-ray exposure to the tongue, for D-erythrose and sucrose was determined. The pre-irradiation threshold value for D-erythrose was found to be 0.02%, confirming the reports that this four carbon sugar is approximately one-tenth as sweet as glucose. There was a delay in the radiation response to local tongue irradiation as compared to the post-radiation saccharin response. The post-irradiation taste acuity for D-erythrose demonstrated only a slight decrease by six hours; by 24 hours a slight improvement in the taste sensitivity was noted. The threshold values had returned to normal pre-irradiation levels at 36 hours.

The pre-irradiation threshold value for sucrose was found to be .0015%. There was an immediate response to local tongue irradiation of 50 R x-ray as compared to the post-radiation saccharin response. The post irradiation taste acuity for sucrose demonstrated a marked decrease by two hours; no improvement in the taste sensitivity was noted until 36 hours post-irradiation. The threshold values returned to normal pre-irradiation levels at 72 hours. This result adds tentative support to our previous hypothesis that molecular size is important in determining the extent of radiation injury to taste acuity. Only four animals were used in this initial experiment.

9. To Determine the Effect of In utero X-Irradiation on Taste Function in the Adult Rat

There was a significant difference in the surgical survival rate following hypothalamic electrode implantation in rats who have received in utero irradiation, no difference in their taste threshold for saccharin could be detected before and after tongue irradiation. Naturally, this experiment was carried out in only 3 animals and a larger group will be necessary to prove this initial impression. It has also been our experience during the past eight years that approximately 90% or animals that survive electrode surgery will work for hypothalamic stimulation and can be trained to obtain a taste threshold. Yet only 50% of the six animals that survived surgery

after in utero irradiation could be trained in our experiment. Therefore, there may be a significant difference in the effect of hypothalamic stimulation in animals that receive in utero irradiation. Again, more animals would be necessary to prove this initial finding.

10. To Determine the Effect of Ingestion of Heavy Metals on Taste Acuity and the Response of Taste Sensitivity to X-irradiation

Oral administration of metals have proven to be effective in improving taste acuity in several disease produced hypogeusic states in man. We tentatively demonstrated a protective action against the radiation effect on taste with these materials which may help to explain the mechanism of radiation action. Six animals were treated with subcutaneous doses of nickel acetate before and after radiation exposure. Six animals acted as controls and did not receive the nickel acetate. It appears that a dose of 0.2 mg. per day of nickel acetate did increase taste acuity in normal non-irradiated animals for quinine hydrochloride. This finding has not been described before, also there does appear to be a protective effect of subcutaneous nickel acetate against radiation injury on the tongue.

3. Graduate Students Trained, Degrees Granted, and Post-Doctoral Tenures Completed

Student Trainees under Supervision of Dr. Gary S. Shaber

Name	Date	Research Project	Present Position	Degree
Dorn, Barry	1963-1965 (Summer)	Taste thresholds	Resident in Surgery, New England Medical Center	M.D. Jefferson Medical College 1966
Koniver, Garth	1965-1966 (Summer)	Taste thresholds	Resident in Radiology, Jefferson Medical College Hospital	M.D., Jefferson Medical College 1967
Penn, Marian	1966 (Summer)	Taste thresholds	Second-year law student, Harvard Law School	B.S. Pembroke 1968
Brent, David L.	1967 (Summer)	Sensory effects of radiation	First-year medical student, Jefferson Medical College	-
Leung, Christopher	1968-1969	Labelling techniques	Instructor in Anatomy, Jefferson Medical College	Ph.D., Jefferson Medical College 1969
Hefton, Jack	1968	Labelling techniques	Doctoral candidate Jefferson Medical College	-
Borthwick, Thomas	1968-1970	Chorda-tympani monitoring	Senior Medical Student, Jefferson Medical College	-
Guralnick, Jack	1969-1971	Taste thresholds	Junior Medical Student Jefferson Medical College	-

Name	Date	Research Project	Present Position	Degree
Grossman, Norman	1967 (Summer)	Taste thresholds	Intern, Jefferson Medical College Hospital	M. D., Jefferson Medical College 1969

4. Bibliography with Titles of Publications Associated with This Contract

A. Publications and Published Abstracts

Shaber, G.S., Rumsey, J.A., Dorn, B.C., and Brent, R.L.: "Saccharin Behavioral Taste Threshold in the Rat." Federation Proceedings 26: 543, 1967 (Abstract).

Shaber, G.S., Hancock, N., Newingham, G., and Brent, R.L.: "Fibrinogen Turnover in the Rat after Whole-Body X-Irradiation." Radiation Research 31: 1967 (Abstract).

Brent, R.L., Hancock, N., Hefton, J., and Shaber, G.S.: "Autoradiographic Localization of Teratogenic Antiserum." American Pediatric Society, 1967 (Abstract).

Shaber, G.S., Brent, R.L., Rumsey, J.A., and Newingham, G.: "The effects of Low Doses of Ionizing Radiation on Taste Thresholds." American Society of Zoologists, 1967 (Abstract).

Shaber, G.S., Brent, R.L., Rumsey, J.A., and Newingham, G.: "The Effects of Low Doses of Ionizing Radiation on Taste Thresholds." American Ecological Society, 1967 (Abstract).

Shaber, G.S., and Brent, R.L.: "Condition-Suppression Behavioral Taste Thresholds in the rat". J. Comparative and Physiol., Psychology 73: 193-201, 1970.

Shaber, G.S.: "Alteration of Taste Thresholds in the Rat Following Low Dose X-Irradiation." Radiation Research 47: 689-703, 1971.

B. Paper Presentations by Principal Investigator

"Saccharin Behavioral Taste Thresholds in the Rat." Federation American Society for Experimental Biology, April 1967.

"Fibrinogen Turnover Following X-Irradiation." Radiation Research Society, May 1967.

"Effects of Irradiation on Saccharin Taste Thresholds in the Rat." American Association for the Advancement of Science, invited symposium, December 1967.

"Alteration of Taste Thresholds by Low Doses of Ionizing Irradiation." Fourth International Congress of Radiation Research, June 1970.

C. Visiting Lectureship
Florida State University, Tallahassee, Florida, 1968.