EIGHTH YEAR PROGRESS REPORT
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TITLE: Alteration of Taste Thresholds by Low Doses of Ionizing Irradiation.

(Report covers period from May 1, 1971 to May 1, 1972)

I. The goals for the eighth year, as outlined in the previous renewal request, included the following:

1. Correlate taste sensitivity change produced by X-irradiation with alteration in diffusion of taste material and transmission at the neurosensory junction.

2. Determine the effect of 150 R of in utero irradiation on adult taste sensitivity.

In addition, work was to continue as follows:

1. Determination of the effects of a combination of irradiation and topical anesthetics.

2. To consider:

a. The following approach to reduce the likelihood of spurious or ambiguous results:

1. Threshold "unstressed";
2. Threshold after agent A;
3. Threshold after radiation
4. Threshold after radiation plus A.

Where agent A is some reasonable envioronmental stress-agent already reported in the literature that produces the taste discrimination effect. These results will be repeated with animals under our experimental conditions.

b. The hypothesis that:

1. Agent A does not affect discrimination; refute by testing (1) and (2).

DISCLAIMER

2. Radiation does not affect discrimination; refute by testing (1) and (2).

3. Radiation does not, when combined with A, affect discrimination; refute by testing (1) and (4).

II. Accomplishments

1. Work Completed During The Eighth Year

Attempts at verification of the sucrose taste threshold before and after X-irradiation were unsuccessful. No apparent reason for this inability in experimentation could be found in spite of the use of sixteen animals in this portion of the experiment. There was statistical fluctuation in the observed thresholds for sucrose between the sixteen animals and in addition there was a daily threshold fluctuation within each animal. It was impossible to obtain an accurate accessment of the radiation effect on the threshold for this taste material. This failure is intriguing, however, a great deal of time was consumed in the pursuit of this threshold and further study had to be suspended so as to proceed with other portions of the experiment. The threshold values for sucrose obtained during the seventh year will have to be considered uncertain and perhaps spurious. In addition, because of the inability to obtain a sucrose threshold the remainder of the work in providing that molecular size is important in determining the extent of radiation injury to taste acuity was suspended.

The use of topical anesthetics to block the neurosensory junction in combination with radiation to the tongue was undertaken. Animals were treated by oral perfusion with 1% procaine solution twenty minutes prior to taste threshold determinations. The duration of treatment with the anesthetic solution was adjusted to produce a ten-fold decrease in taste acuity. There was a considerable daily fluctuation in the duration of treatment time necessary to achieve this end point. This may be due to a variability in application of the anesthetic or to a variation in individual animal response to topical anesthetics when applied to the tongue. In spite of these difficulties a definite additive effect in reduction of taste acuity was seen when the animals were treated with topical anesthetics and 50 rads of radiation in combination (see Table I). It is uncertain that this result aids in explaining the site of the action of radiation on taste acuity. We still could not be certain that radiation and the topical anesthetics are or are not working at the same sites. Since an additive effect could result from two different sub-maximal depressions of taste acuity acting at different sites. A total of twelve animals were used in this portion of the experiment and saccharin was the taste material.

Recent reports by other authors during the past year have perhaps provided a better means of identifying the site of radiation action on taste. Oral administration of metals have proven to be effective in improving taste acuity in several disease produced hypogeusic states in man. If we could demonstrate a protective action against the radiation effect on taste with these materials we could then explain the mechanism of radiation action. It was for this reason that six animals were treated with subcutaneous doses of nickel acetate before and after radiation exposure. Half of the animals acted as controls and did not receive the nickel acetate. Several interesting experimental results were obtained during the course of the experiment. 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. There does appear to be a protective effect of subcutaneous nickel acetate against radiation injury on the tongue (See Table II). More animals will be used to verify this result.

Techniques that had been established during the seventh year for producing in utero radiated animals with shielded contra-lateral horn siblings to act as controls were applied in determining the effects of in utero radiation on the animals taste acuity when an adult. The fetuses were delivered by caesarean section one day prior to term and then given to foster mothers who delivered one day before. At the time of caesarean section the fetuses were marked by clipping their tails. This was the only way that controls could be separated from the irradiated animals. Acceptance of the radiated animals with the foster mothers was somewhat unpredictable however, a 50% success rate of acceptance did occur. Several litters of animals were prepared in this manner and electrode implantations in the hypothalamus were attempted. Unfortunately mortality rate of this surgery was exceptionally high in the radiated animals. It was uncertain whether the dose of radiation, 150 R on the eighteenth day of gestation, was responsible for this high surgical mortality or due to the stock of animals used. In our other experiments we had been experiencing high operative mortality using the stock of animals presently housed in the Stein Research Center. There is a high incidence of respiratory infection and ear mites in these animals. It was necessary for us to procure animals from outside sources and once again we are able to achieve a 95% survival rate following hypothalamic electrode insertion. It was for these reasons that several pregnant animals were obtained from animal supply houses and 100 rads of radiation was delivered to the fetuses on the eighteenth day of gestation. It is hoped that a combination of different stock and a lower radiation dose will permit us to successfully implant hypothalamic electrodes when the animals reach maturity. Two of these litters have delivered and the animals now

weigh 175 grams, approximately 25 grams less than necessary for hypothalamic electrode implantation. We expect to insert these electrodes within the next two to three weeks. As soon as the electrodes are implanted we then can begin the initial pilot portion of the experiment concerned with the effects of in utero X-irradiation on adult taste thresholds. Controls for this experiment will be drawn from our previous taste threshold work. In addition, these animals will be studied to determine if there is an alteration of the hypothalamic self-stimulation rates following in utero irradiation. This latter portion of the experiment will be necessary to determine if the behavioral motivating system is intact following in utero irradiation. Again, controls will be provided by our previous experience with normal animals that we have used the proceeding eight years of this project.

2. Objectives for the Remainder of the Eighth Year

During the remainder of the eighth year of our work on this project the pilot portion of the in utero radiation effects will be completed. 100 R of X-irradiation on the eighteenth day of gestation to the fetus is the radiation dose, and Wistar rats obtained from outside sources are the experimental animals. Electrodes will be implanted in the in utero irradiated animals that are now reaching maturity. Also, the effects of head only irradiation on hypothalamic stimulation rates will be determined. Doses of 300, 600, 900, 1200, 1500, and 1800 rads will be given to the head and the hypothalamic stimulation rates before and after the exposure will be compared to our earlier work using normal non-in utero irradiated animals. If significant changes are observed then litters with contra-lateral control horn mates will be used to substantiate these preliminary results.

In addition, further experimentation with the protective effects of nickel acetate on radiation effect will continue.

3. Summary

A. Subcutaneous "loading" of animals with nickel acetate seems to lower taste thresholds for quinine hydrochloride in normal rats.

B. Subcutaneous nickel acetate given before and after 50 rads of tongue X-irradiation seems to lessen the radiation effect.

III. General Summary

A. Travel

1. Attendance at the Radiological Society of North America meeting, Chicago, Illinois, December, 1971.

2. Attendance at the Faseb Meeting, Atlantic City, New Jersey, April, 1972.

B. Publications

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

2. Shaber, G.S., and Brent, R.L.: Fibrogen Turnover in the Rat after Whole-Body X-Irradiation. Part II. The Effects of Dose and E-Aminocaproic Acid. in preparation.

Table I

Effect of Radiation in Combination With 1% Procaine

on Saccharin Taste Threshold

(Grams per 100 ml.)

Animals	TASTE THRESHOLD					
	Before Radiation	Days Post Radiation				
		1	2	3	4	5
Controls	.001	.001	.001	.002	.002	.0005
Radiation Only	.001	.01	.005	.003	.003	.001
Procaine Only	.01	.01	.02	.008	.008	.000
Radiation and Procaine	.01	.015	.015	.01	.01	.01

Table II

Effect of Radiation in Combination With Nickel

Acetate on Quinine Hydrochloride Taste Threshold

(Grams per 100 ml.)

Animals	TASTE THRESHOLD					
	Before Radiation	Days Post Radiation				
		1	2	3	4	5
Controls	.00015	.00010	.00020	.00015	.00015	.00010
Radiation Only	.00010	.00100	.00020	.00020	.00010	.00010
Nickel Acetate Only	.00005	.00010	.00005	.00015	.00001	.00005
Radiation and Nickel Acetate	.0001	.00030	.00010	.00005	.00010	.00010