7. Troll, W., Belman, S., Nelson, N., PROC. SOC. EXP. BIOL. AND MED., (in press).
8. Grandmougin, E., *Ber. Devt. Chem. Ges.*, 1906, v39, 2494.
9. Boyland, E., Manson, D., *Biochem. J.*, 1957, v67, 275.
10. Folin, O., *J. Biol. Chem.*, 1922, v51, 377.
11. Levvy, G. A., *Biochem. J.*, 1952, v52, 464.
12. Booth, J., Boyland, E., Manson, D., *ibid.*, 1955, v60, 62.
13. Dische, F., *J. Biol. Chem.*, 1947, v167, 189.
14. Troll, W., Nelson, N., *AIHA Journal,*
15. Clayson, D. B., Jull, J. W., Bonser, G. M., *Brit. J. Cancer,* 1958, v12, 222.

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Effects of Prenatal X-Irradiation on Discrimination Learning in the Rat. (24530)

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It is well established that x-irradiation during prenatal development may result in death, retarded growth and morphological abnormalities, the exact type and magnitude of the effect depending on embryonic stage irradiated and dose used [see review by Russell (1)]. Prenatal x-irradiation also affects maze learning performance of rats. Levinson(2) irradiated rats on 11th to 19th days of pregnancy with single sublethal exposures of x-irradiation and tested offspring when 50 days old on Lashley Type III maze. Learning measured in terms of number of trials to reach a criterion, number of errors and time spent in maze, was impaired with deficits directly related to radiation dose. Radiation on 13th day produced greatest changes. This agrees roughly with Hick's timetable for cortical damage (3). Similar findings were also reported by Tait *et al.*(4) and more recently by Furchtgott *et al.*(5). Data are here presented on effects of x-irradiation during various stages of prenatal development (10th, 14th and 18th day of pregnancy) on behavioral performance on another type of task, *i.e.*, learning a brightness discrimination.

Procedure. Fifty female rats of Long-Evans strain, raised from weaning on natural food stock ration,‡ were selected at 3 to 4 months of age and average body weight of 188 g. Animals were divided at random into 5 equal groups of 10 rats each and mated to males of proven fertility. Pregnancy was dated by finding sperm in vaginal smear.§ Animals in Group I served as non-irradiated controls: those in Groups II, III and IV received a single exposure of 150 r x-irradiation on 10th, 14th or 18th day of pregnancy respectively. Group V received a single exposure of 300 r on 18th day of pregnancy.‖ Radiation factors were as follows: GE Model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic fil-

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§ Start of pregnancy was dated from time that sperm was actually observed in vaginal smear. Inasmuch as mating may have occurred at any time from 5 p.m. of the previous day to 8 a.m. of the day sperm were found, fertilization may have occurred as much as 15 hours before the time that sperm were observed. First day of pregnancy covered the first 24 hours following finding of sperm. It is estimated that actual time of x-irradiation in our experiment was approximately 9½, 13½ and 17½ days post fertilization.

‖ Two additional groups of 10 rats each receiving a single exposure of 300 r x-irradiation on 10th and 14th day of pregnancy were also included. No litters were born to any rats in 10 day 300 r group. In 14 day 300 r group, 7 litters were born but all young died within 5 days of birth.

ter;¶ HVL, 2.15 mm Cu; target distance to top of box, 78 cm; and exposure dose rate, 17.92 r/minute (measured in air, at top of box). Animals to be irradiated were placed in wooden box divided into 14 compartments 7 cm wide, 16 cm long and 10 cm deep. Partitions and top were made of 1/8" cellulose acetate sheeting; and top, one side and bottom of each compartment were perforated with holes for ventilation. The container was rotated slowly on electrically-driven turntable to insure equivalent irradiation. Throughout pregnancy and lactation, rats were continued on same ration they had received since weaning. Food and water were provided *ad lib*. In Groups I to V, proportion of females casting litters was 90%, 100%, 100%, 90% and 80%; average litter size was 7.8, 7.6, 9.3, 7.7 and 7.3; average birth weight of young, 6.1 g, 5.4 g, 5.5 g, 5.6 g and 5.2 g; and mortality of young during lactation, 7.0, 39.5, 9.6, 9.8 and 13.0%, respectively. Thus, the group varied significantly only in the last parameter. At weaning the young appeared normal except for offspring of group II. The latter in accordance with previous findings(1) exhibited a number of abnormalities including microphthalmia and anophthalmia (present in approximately 40% of group), hydrocephalus (grossly discernible in approximately 20%), paralysis of hind limbs (noted in approximately 10%) and shortening and kinking of tail (approximately 10%). At weaning 10 female rats of comparable size were selected from offspring of Groups I to V respectively. These consisted of at least one animal from each litter weaned. The offspring selected from Group II were carefully screened to eliminate those with grossly detectable defects. Group designations A, B, C, D, E have been applied to young from Groups I to V respectively. These rats were continued on previous diet until approximately 180 days of age, then subjected to a brightness discrimination problem. Body weight of rats in the various groups at this time was as follows: 261 g; 246 g; 209 g; 224 g; and 226 g respectively.

Training procedure. The task was to solve a brightness discrimination problem in which a dim light in the box indicated that the animal could drink freely; a bright light indicated that the drinking tube was electrified and animals attempting to drink would receive a shock. Preliminary to actual training all animals were placed on 23-hour water deprivation schedule. For 2 days they were allowed to drink in the apparatus for one hour *ad lib*, when the signal light would remain dim. During the learning phase the signal light would alternate between periods of low (dim light) and high (bright light) intensity. Dim light indicated a non-shock period while bright light indicated a shock period. Thus, the animal received shock only if it drank while the bright light was on. The bright light preceded the shock phase by 2 seconds. The time sequence control was set for a 10-minute cycle of alternating periods of shock and non-shock for the following minutes: 2, 1, 1, 3, 2, 1. This cycle was repeated 6 times during the training hour. Each day's training began at a different point on the cycle to insure against the animal learning the sequence. Animals remained in box for one hour each day, until they reached and maintained at least 80% correct responses for 2 consecutive days. Any drinking during a non-shock period was considered a correct response, while drinking or attempt to drink during a shock period was classified as incorrect response. Animals were tested daily for 12 consecutive days.

Apparatus. A training compartment 9" wide, 8" high and 14" in length was constructed of 3/4" plywood. The floor was covered with a single wire grid resting 1/2" above floor of box. Entrance to training compartment was a hinged door with a wire observation window covered during training trials to make the box light tight. A 1" diameter signal light with a white frosted filter was located in middle of wall opposite the entrance, 3" below top of compartment. On the same wall and 3" directly below signal light, a 1/2" copper drinking tube entered the com-

¶ A non-uniform filter which produces a flat isodose surface of x-ray intensity constructed by method of Greenfield and Hand(6). We are indebted to Dr. M. Greenfield and Katherine Hand of Atomic Energy Project, Univ. of California at Los Angeles, for construction of parabolic filter. Center of filter had thickness of 1.7 mm Cu; the edge, 0.5 mm Cu.

partment. A 250 cc water bottle was suspended outside the compartment behind back wall. Brightness of signal light was controlled by a rheostat. The copper drinking tube was electrified in such a fashion that when charged the animal received a shock when he completed the circuit from the grid on the floor to the copper drinking tube. The current was controlled by an electronic constant current device. A current of 0.4 milliamperes was used. Recording of drinking was accomplished by a 6-volt circuit which registered the breaking of mechanical contacts when drinking tube was touched. Time sequences for shock and non-shock periods were controlled by a synchronous motor driven cam and microswitches.

Results. The performance of rats in various groups in terms of correct responses is summarized in Fig. 1. The offspring of non-irradiated rats (Group A) reached criterion (minimum of 80% correct responses for 2 consecutive days) on day 9. The offspring of rats administered a single dose of 150 r on 10th or 18th day of pregnancy (Groups B and D) reached criterion a day later (i.e., 10th day). Offspring of rats administered 150 r on 14th day of pregnancy (Group C) or 300 r on 18th day of pregnancy (Group E) had not yet attained criterion after 12 days of testing. On 13th day the latter 2 groups were exposed to absolute discrimination problem (*i.e.*, presence *vs.* absence of light) and the tests continued for additional 4 days. Criterion was still not attained by rats in these groups after the additional 4 days under these conditions.

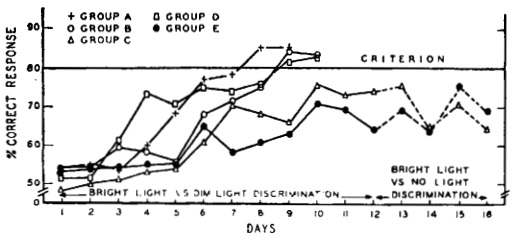

FIG. 1. Discrimination learning performance of rats in Groups A-E. Group A, offspring of non-irradiated rats. Groups B, C and D, offspring of rats which received a single exposure of 150 r x-irradiation on 10th, 14th or 18th day of pregnancy respectively. Group E, offspring of rats administered single dose of 300 r x-irradiation on 18th day of pregnancy.

TABLE I. Means and Stand. Dev. of Errors for Experimental and Control Groups on Day 10 (N = 10 for All Groups).

Groups	Mean	Stand. dev.
A	2.5	1.02
B	3.1	1.37
C	6.1	1.45
D	3.5	1.50
E	6.6	1.49

TABLE II. Analysis of Variance of Error Scores for All Groups.

Sources of variation	df	Variance	F
Between groups	4	34.58	16.31*
Within "	45	2.12	
Total	49		

* Significant at .001 level of confidence.

An error was defined as inappropriate response to the stimulus, *i.e.*, attempting to drink during the bright light (shock) phase of a trial. A trial consisted of a dim light-bright light sequence. There were 18 such sequences a day. A summary of mean number of errors for various groups during the regular 1 hour training period on day 10 (when offspring of non-irradiated rats met criterion) is presented in Table I. A one way analysis of variance of the number of errors on day 10 was performed. Table II summarizes results of analysis of the data.

An F-ratio computed on data, proved to be highly significant. That no significant differences existed between means of 3 groups that met criterion (A, B, and D) is borne out by a t-test of the difference between means of Groups A and the poorer of the 2 irradiated groups that met criterion (*i.e.*, Group D). Since the learning problem involved discriminating between bright and dim light, the possibility arose that a poor performance might reflect not impaired capacity to learn but rather impaired visual function. The latter is particularly applicable to offspring of Group II since microphthalmia and anophthalmia occurred in approximately 40% of animals in this group and it is possible that ocular defects were present in Group B which were not apparent by gross inspection. Since these rats attained criterion however, on the same day as animals in Group D and only a day after the offspring of non-irradiated controls

(Group A), it is apparent that any ocular defects that may have been present in rats in this group were not sufficiently marked to impair their learning the discrimination problem. The slightly poorer performance of rats in Group B during the early phase of learning (Fig. 1) as compared to that of rats in Groups A and D may have been due to such defects. Additional evidence that the learning performance of rats in Group B was not genuinely lower than that of Group D is indicated by the error score of rats in the 2 groups on day 10 (Table I).

Present findings indicate that prenatal x-irradiation significantly altered behavioral performance of rats exposed at 6 months of age to a brightness discrimination problem. The effects obtained were dependent on stage of pregnancy when x-irradiation was administered and dosage employed. After a single dose of 150 r, discrimination learning performance was impaired only in offspring of rats irradiated on 14th day of pregnancy. After a dose of 300 r, discrimination learning performance was impaired in offspring of rats irradiated on 18th day of pregnancy as well. These observations are in accord with previous findings that prenatal x-irradiation impaired the maze learning performance of rats (2,4,5) and provide additional evidence that sublethal doses of x-irradiation during the latter trimester of pregnancy may result in a learning decrement in the rat.

Summary. Offspring of non-irradiated rats which received a single dose of 150 r x-irradiation on 10th, 14th or 18th day of pregnancy respectively or 300 r on 18th day of pregnancy were exposed at approximately 6 months of age to a brightness discrimination problem. Data were evaluated on the basis of number of trials to criterion and number of errors for the various groups on the day when offspring of non-irradiated group reached criterion. Findings indicate that a single exposure of 150 r on 14th day or 300 r on 18th day of pregnancy significantly impaired discrimination learning performance as compared to the performance of non-irradiated rats or offspring of rats administered a single dose of 150 r x-irradiation on 10th or 18th day of pregnancy.

1. Russell, L. B., *Radiation Biology,* Edit. by A. Hollaender, McGraw-Hill Book Co., N. Y., 1954, Chap. 13.

2. Levinson, B., *J. Comp. Physiol. Psychol.,* 1952, v45, 140.

3. Hicks, S. P., *J. Cellular Comp. Physiol.,* 1954, v43, Suppl. 1, 151.

4. Tait, C. D., Jr., Wall, P. D., Balmuth, M., Kaplan, S. J., *USAF Sch. Aviat. Med. (Spec. Rep. No.* 11), 1952.

5. Furchtgott, E., Echols, M., Openshaw, J. W., *J. Comp. Physiol. Psychol.,* 1958, v51, 178.

6. Greenfield, M. A., Hand, K., *Am. J. Roentgenol.,* 1952, v68, 960.

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Inhibition of Adrenal Medullary Responses to Chemical Stimulation.* (24531)

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Hexamethonium antagonizes the ganglionic stimulation produced by acetylcholine and other depolarizing substances such as nicotine or tetramethylammonium(1). The adrenal medulla is in many ways analagous to a sympathetic ganglion and is known to respond to ganglionic stimulating agents by release of medullary hormones(2). However, the influence of ganglionic blocking agents on responsiveness of adrenals to direct chemical stimulation has not been adequately described. In this study, experiments have been carried out to determine effects of hexamethonium and of

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