of the reduction in systemic arterial pressure(7).

The stop flow experiments present evidence that diazoxide does not act on the distal tubule to inhibit sodium transport since diazoxide did not elevate the distal stop flow sodium minimum concentration. It appears, therefore, that the natriuresis which occurs when diazoxide is given directly into the renal artery is probably due to renal vasodilation resulting in an increase in renal blood flow, glomerular filtration rate and alterations in intrarenal hemodynamics. That the hemodynamic alterations are the major cause for the natriuresis is supported by the fact that no distal tubular effect on sodium transport could be detected using the stop flow technique.

Summary. Renal clearance and stop flow studies were done in dogs before and after infusion of diazoxide directly into the left renal artery. In all animals the left kidney showed a prompt diuresis, natriuresis and increase in creatinine or inulin clearance when diazoxide was given. No inhibition of distal tubular sodium reabsorption following diazoxide administration was found using the stop flow technique. The natriuresis produced by infusing diazoxide directly into the renal artery is probably due to renal vasodilation and resulting changes in intrarenal hemodynamics.

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Uterine Metabolism Changes During Gestation in Rats of Different Age Groups.*† (32096)

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Reduction of triphenyltetrazolium chloride (TTC) by uterine tissue of rats apparently measures the estrogen status of the uterus since the rate of reduction is increased in proportion to estradiol dosage in spayed females and is highest at pro-estrus in intact cyclic females. Furthermore, the effect of estradiol on uterine TTC reduction rate is diminished by progesterone in proportion to the progesterone dosage(1,2).

Using TTC reduction rate of uterine tissue as a metabolic index, Schultze reported that maximum litter size in rats was associated with a level of uterine metabolism that varied during different stages of gestation(3). Size of litter is influenced by many factors of which intrauterine mortality is an important one. Intrauterine mortality reportedly increases in old rats(4,5). If reduced litter size in old

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TABLE I. Effect of Stage of Pregnancy and Age on Uterine TTC Metabolism.

Age Group	day 0		day 4 post-breeding		day 8 post-breeding		day 12 post-breeding		day 16 post-breeding	
	No. Rats	TTC units* Mean ± S.E.	No. Rats	TTC units Means ± S.E.	No. Rats	TTC units Mean ± S.E.	No. Rats	TTC value Mean ± S.E.	No. Rats	TTC units Mean ± S.E.
60-80 days	12	119 ± 5.2	12	88 ± 4.0	12	80 ± 3.5	12	96 ± 3.6	12	88 ± 4.2
120-150 "	12	115 ± 5.0	12	95 ± 4.1	12	85 ± 3.0	12	84 ± 3.2	12	82 ± 4.3
300-330 "	12	112 ± 4.5	12	83 ± 4.4	12	75 ± 3.2	12	90 ± 3.0	12	91 ± 3.5

* 1 unit TTC reduction = 0.01 μg formazan produced per 1 mg wet uterine tissue/hour.

females is due principally to increased fetal loss during gestation, and if a varying estrogen status of the uterus (TTC reduction) is related to maximum litter size, then old females would be expected to have a different uterine estrogen status at some stage or stages of gestation than younger females that produce larger litters.

It was the purpose of this study to determine whether different age groups of female rats had levels of uterine TTC metabolism at various stages of gestation that differed and whether any differences might account for intrauterine losses.

Materials and methods. Rats of Wistar origin, maintained in this laboratory, were used. The animals were 60-80 days of age; 120-150 days of age; and 300-330 days of age when bred. The following data were recorded and studied for reproductive performance: number of corpora lutea, implantation sites, and living fetuses at day 8, 12, and 16 post breeding. Corpora lutea were recorded for each of 12 females in each group at days 8, 12, and 16 post-breeding when the living fetuses at these stages were recorded. Implantation sites were recorded for each of 12 females in each group at days 12 and 16 post breeding. Also implantation sites were recorded for 24 females that were allowed to litter in the 60-80-day-old group and in the 120-150-day-old group and for 15 females allowed to litter in the 300-330-day-old group. Litter size at term was recorded in the latter 63 females. Uterine tissue reduction rate of triphenyltetrazolium was determined at day of breeding (day 0) and at 4, 8, 12, and 16 days post-breeding. Twelve females for each of these days and for each age group were used in these determinations. The method used to determine uterine TTC reduction rate has been described previously(1). Statistical study of the TTC reduction rate data was made by an analysis of the variance. Differences in means of TTC metabolism for stages of gestation and between age groups were tested for significance. The data on reproductive performance were studied for significance of differences between age groups for intrauterine losses from ovulation (corpora lutea) to implantation, and for losses from implanatation to littering.

Results. Uterine TTC reduction rate at different stages of gestation (Table I). In all age groups, uterine TTC metabolism decreased significantly from day 0 to 8 post-breeding. The 300-330-day-old group had a significantly lower uterine metabolism at day 4 and day 8 post-breeding than did the 120-150-day-old females ($P<.01$). From day 8 through day 16 post-breeding uterine metabolism in the 120-150-day-old group was rather constant. In the 60-80-day-old group and the 300-330-day-old group, there was a significant increase ($P<.01$) in uterine metabolism at day 12 compared to that at day 8 post-breeding. This higher rate was maintained through day 16 post-breeding in the 300-330-day-old females but not in the 60-80-day-old females.

Reproductive performance in varying aged rats (Table II). Ovulation rate (number of corpora lutea) and implantation number were somewhat higher in the 120-150-day-old females than in the other age groups but differences were not significant. Intrauterine loss from ovulation to implantation was not significantly different between age groups. Fetal loss from implantation to term is significantly greater in the 60-80-day-old females than in

TABLE II. Reproductive Performance Data in Varying Aged Rats.

	Corpora lutea		Implantations		Living Fetuses day 8		Living Fetuses day 12		Living Fetuses day 16		Litter Size		Loss from implantation to term	
	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.
60-80 days	36	16.3 ± .45	48	14.4 ± .60	12	14.1 ± .66	12	13.3 ± .56	12	12.8 ± .56	24	12.2 ± .66	24	2.2 ± .39
120-150 days	36	17.3 ± .44	48	15.2 ± .47	12	15.0 ± .62	12	14.7 ± .56	12	14.7 ± .55	24	14.3 ± .55	24	0.9 ± .28
300-330 days	36	16.5 ± .46	39	14.9 ± .57	12	14.9 ± .67	12	13.1 ± .67	12	12.2 ± .70	15	11.1 ± .66	15	3.8 ± .55

the 120-150-day-old females ($P<.01$). Fetal loss during the same period of gestation was significantly greater in the 300-330-day-old females than in the 120-150-day-old group ($P<.01$). The 300-330-day-old group also showed a greater fetal loss from implantation to term than the 60-80-day-old group ($P = .01$).

Reproductive loss from ovulation to littering was about 25% in the 60-80-day-old females; 18% in the 120-150-day-old females; and 33% in the 300-330-day-old females.

Discussion. Within the age range studied, it appears that differences in litter size between age groups is mainly due to fetal losses during the latter half of gestation and not to differences in ovulation rates or implantation rate. Increased uterine TTC metabolism at about midpregnancy occurred in those age groups with the greater fetal loss during the latter half of gestation. A rise in uterine TTC metabolism has been shown previously to result from an increased estrogen or a decreased progesterone stimulus in relation to a constant estrogen stimulus(2). It is, therefore, thought that in the young and old females either an increased release of estrogen or a decreased amount of progesterone in relation to estrogens occurred at about midpregnancy. Zarrow(6) reported decreased gestagens in mice about midpregnancy. A similar situation may be present in the old and young rats in this experiment. In the rat the placenta secretes a luteotrophic hormone beginning about the tenth day of gestation(7) and a deficiency in this mechanism would lead to decreased progesterone and would be reflected in an increase in uterine TTC reduction rate if estrogen secretion remained unchanged. A relative lack of progesterone at midpregnancy would account for the increased fetal loss in the young and old females during the latter half of gestation.

The reason that a change in estrogen to progesterone ratio occurred near midpregnancy in old and young females but not in females in the prime of reproductive life can only be speculated on. Possibly in young females with a uterus of limited size and in old females with decreased lability of their

uterine tissue(8,9) a change in the relationship between estrogen and progesterone is induced in an attempt to facilitate uterine accommodation of the developing fetuses(10) but in facilitating accommodation, underproduction of progesterone if proceeding too far results in increased fetal loss.

Summary. Change in the TTC reduction rate of uterine tissue during gestation in varying aged rats was determined. Old females (300-330 days) and young females (60-80 days) showed an increase in uterine TTC reduction rate at day 12 post-breeding, whereas females 120-150 days old did not. Fetal loss was greater in the 300-330-day-old and the 60-80-day-old females than in the 120-150-day-old females. The stage of gestation when increased fetal losses occurred appeared to coincide with the rise in uterine TTC reduction rate at day 12 post-breeding.

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Behavioral and Structural Changes Following X-Irradiation of the Forebrain in the Rat.* (32097)

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Experimental findings have been consistent in showing learning deficits for the radiosensitive prenatal and very young postnatally irradiated rats but the results of radiation studies in the adult animal have been generally negative. Previous studies have demonstrated that neither non-lethal doses of whole body radiation nor doses up to several thousand R to the brain adversely affect learning in older rats whenever abscopal effects or motivational changes do not inhibit performance(1-4). Arnold(3) did observe, however, that x-irradiation had a long-term deleterious effect on discrimination learning. His experimental rats, which had received 5,000 R to the brain 323 days prior to testing, made significantly more errors in a 2-choice discrimination box than did the controls. Since it is well established that x-irradiation induces changes in the central nervous system of the adult rat(5,6), Urmer and Brown(7) have suggested that the reason for the almost universal failure to detect changes in learning and retention following irradiation was that the problems designed for the animals were not sensitive enough to detect radiation effects.

Recently, it was reported(8) that if a delay in reinforcement is alternated with immediate reward in an alley runway, rats could discriminate between the two sets of trials if delays of 60 seconds or longer were imposed on the delayed trials. Using this procedure, Burt and Ingersoll(9) demonstrated a learning deficit in 65-day-old rats which had been subjected to 5,000 R x-irradiation directed to the frontal area of the cerebrum. The irradiated

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animals were found to be retarded in the acquisition of the alternating pattern of immediate and delayed reward. Examination of the brains of these animals revealed extensive histochemical and histopathological changes in the rostral half of the forebrain(5).

The present investigation was designed to determine the effect of ionizing radiation, directed to the entire cerebrum, upon the acquisition of an alternating pattern of immediate and delayed reward in 90-day-old rats.

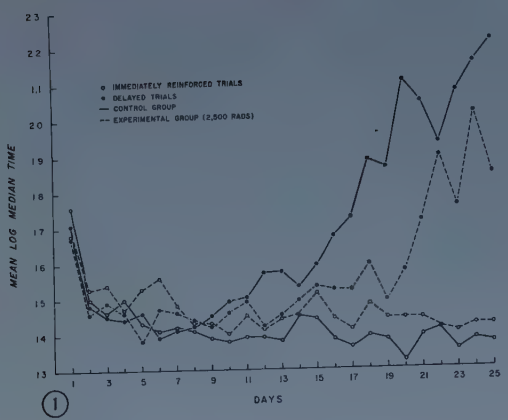
Materials and methods. Forty-five naive, Sprague-Dawley (Holtzman strain) male rats were divided randomly and equally into one control and 2 experimental groups, one of which received 2,500 and the other, 5,000 rads of x-rays to the forebrain. The animals were irradiated using a 250 kvp General Electric Maxitron x-ray machine operating at 250 kv and 30 ma. A 1 mm Al and a 0.5 mm Cu filter resulted in a beam having a dose rate of 490 rads per minute and a half value layer of 2.15 mm Cu. Depth dose measurements were made with lithium fluoride thermoluminescent dosimeters implanted in the forebrain of a sacrificed animal. The rats were anesthetized with intraperitoneal injections of pentobarbital sodium and secured to a lucite holder with their heads immobilized in contact with a 1 cm^2 beam collimeter so that the cerebrum was directly in front of the aperture without exposing the eyes. All controls were similarly anesthetized. All radiation procedures were conducted at Brookhaven National Laboratory before the animals were shipped to the Medical College of Virginia in Richmond for behavioral testing.

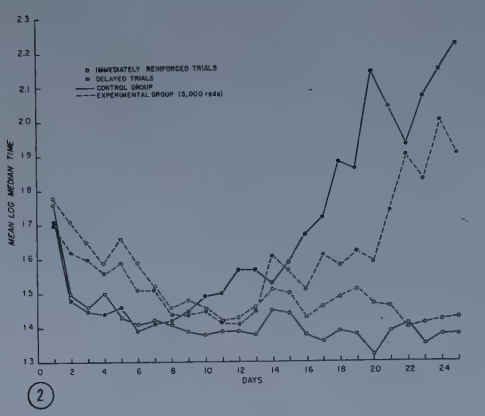
The testing apparatus consisted of an alley runway 6.46 feet in length and was similar to that described elsewhere(8-10). Training began 4 weeks post-irradiation. Data were collected in two replications. The animals were placed on a 23-hour food deprivation schedule one day prior to beginning the studies. Water was available *ad lib* throughout the experiments. All animals were weighed once a week, and to avoid the possible influence of nocturnal cyclic effects, their quarters were lighted continuously. Following each handling period or training trials, a bowl of wet mash made from Purina chow meal was placed in each of the home cages for one hour. On days 1 through 6, each group was handled one hour each day on a large table. On days 7 through 9, each rat was given 4 immediately rewarded trials per day in the alley runway. A 20-second reinforcement period was followed by a 20-second intertrial interval. On the 10th day, the first of 25 consecutive days of acquisition training began. Each animal was given 10 training trials a day. On the even-numbered trials a 20-second immediate reinforcement was given while on the odd-numbered trials each animal was delayed 60 seconds. Running times were taken from a Gra-Lab micro timer, operated by a hand switch. The feeding, delay and intertrial intervals were measured with a stopwatch.

At the conclusion of the experiments the animals were decapitated, their brains removed and fixed in either 10% neutral buffered formalin, Cajal's fluid, or Rossman's fluid. After sectioning at 7 μ, the tissues were stained either with toluidin blue, the Feulgen reaction, Cajal's gold sublimate or PAS-hematoxylin.

Results. At the conclusion of the trials the median running time for the immediately reinforced and for the delayed trials was obtained for each animal for each day. The median score was then converted to its logarithm ($\log_{10}$). The data for the learning curves consist of a plot of the mean log median time for each of the 25 days of trials for each group. These values were obtained by summing the log scores of the immediately reinforced trials of a group for each day and dividing by the number of animals. The same was done for the delayed trials. Examination of the learning curves for the 2,500 rad group (Fig. 1) and for the 5,000 rad group (Fig. 2) revealed that patterned running‡ appeared in the control and 2,500 rad groups about the 10th day of the trials and in the 5,000 rad group about the 14th day, but both irradiated groups exhibited an

‡ Patterned running refers to fast running on the immediately reinforced trials and to slow running on trials with delayed reward.





FIGS. 1 and 2.

inferior performance for the remainder of the trials.

To determine the significance of the learning curves, the data for the last 10 days were subjected to a 2 × 2 analysis of variance(11) with the irradiated *vs* the control groups and immediately rewarded *vs* delayed trials as the main effects. In the analysis of the 2,500 rad group (Fig. 1), Groups, *i.e.*, 2,500 rad group, *vs* Controls ($F = 14.069$, $df = 1/28$), Trials ($F = 289.171$, $df = 1/28$), and Groups × Trials interaction ($F = 38.067$, $df = 1/28$), all were highly significant ($p < .001$). Statistical analysis for the 5,000 rad group (Fig. 2) revealed almost identical results: Groups, *i.e.*, 5,000 rad group, *vs* Controls ($F = 6.553$, $df = 1/28$), Trials ($F = 170.364$, $df = 1/28$), and Groups × Trials interaction ($F = 15.293$, $df = 1/28$), were all highly significant ($p < 0.1$ and $p < .001$).

Examination of the light microscope preparations of the 30 irradiated animals revealed two brains with large lesions. The larger of the 2 necrotic areas occurred in the posterior-inferior portion of the forebrain, involving the ventral hippocampal area and extending to the meninges in an animal which had received 2,500 rads. The necrotic areas were relatively recent, containing macrophages, mononuclear cells and filled with a PAS-positive granular material. Smaller areas of focal necrosis, including some degenerating neurons, were observed scattered about and sometimes interconnected with the edges of the primary lesion. Neurons, in general however, showed little evidence of alteration. The second of the specific lesions was noted in the brain of a rat which had received 5,000 rads. The area of necrosis was similar to the previously described lesion but smaller in area and located farther rostral in the forebrain. Other irradiated brains showed less specific evidence of change, such as loosening of white matter and scattered, small areas of infarction. It was further noted that some blood vessels, capillaries and small arteries in irradiated brains exhibited evidence of modest adventitial hyperplasia and endothelial hypertrophy. The most specific, but diffusely scattered alteration, was in the neuroglia, characterized by hypertrophy and increasing fibrillary nature of astroglia. There was a greater astroglial response at 5,000 than at 2,500 rad dose.

All the animals appeared healthy, were well motivated, and gained weight in a similar manner, as shown in Fig. 3. Statistical analysis using Student's t test indicated that during the training and testing period the mean weights of the 3 groups were not significantly different. However, at the time of sacrifice the irradiated animals did show a significantly lower body weight than did the unirradiated controls ($p < .05$). The lack of early weight differences indicates that in all probability there were no abscopal effects altering the animals' performance in the testing situation.

Discussion and conclusions. The results of the present experiments clearly indicate a retardation in the acquisition of an alternating pattern of immediate and delayed reward. This became evident after the 15th day of

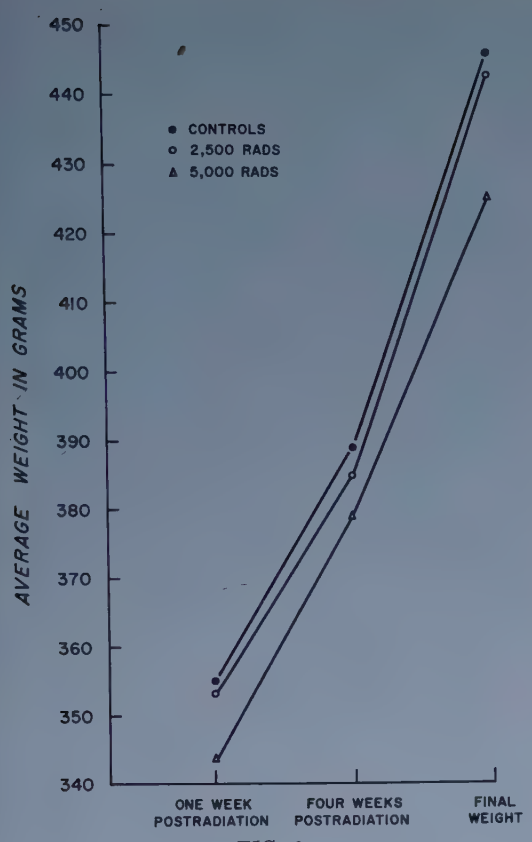


FIG. 3.

the trials in both the 2,500 and 5,000 rad groups (Fig. 1 and 2). This learning deficit is ascribed to the effects of ionizing radiation directed to the forebrain and not due to abscopal effects or motivational factors which affect performance. All animals ran equally well on the immediately rewarded trials. There were no significant differences in the learning curves of the 2,500 and 5,000 rad groups which was not surprising since light microscope examination of the nervous tissue revealed, in general, only minor neuropathological differences in the reaction of the neuronal, glial and blood vascular structures to the 2,500 and 5,000 rad doses.

Nearly all areas of the forebrain were irradiated. The beam included the hippocampus, thalamus, hypothalamus, basal ganglia and all of the cerebral cortex except the most rostral part. Aside from the two brains with large necrotic lesions the remainder of the irradiated brains exhibited moderate alterations in blood vessels and to a limited extent in the underlying white matter. Astroglial hypertrophy was a consistent finding in all irradiated brains.

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