mice used in this study(7) show no increase in mortality in irradiated line mice between live birth and weaning. It would appear from the results of this and other studies reported here that, although the theory of reduced viability (life span shortening) as a measure of increased mutation rate is a logical one, factual statements to this effect may be premature. Studies continue, however, and offspring and grandoffspring from 25, 30, and 35 generations of paternal X-ray exposures are now being studied.

Summary. Life span studies on first generation male and female breeders and on second generation virgin females from 20 generations of X-irradiated sires were done. The results of this and other studies strongly suggest that factual statements crediting reduction of life span as being a measure of a radiation-induced genetic decrement in viability are premature and inconsistent with experimental evidence at this date.

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Plasma Levels of Ovarian Ascorbic Acid Depleting Activity (Luteinizing Hormone?) in Pregnant Rabbits.* (30338)

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Recent interest in the action of pituitary luteinizing hormone (LH) in enhancing the *in vitro* synthesis of progesterone in slices of luteal tissue(1) and its possible luteolytic role under certain conditions(2) make a systematic study of plasma LH levels in pregnant animals especially timely. The rabbit, in which ovulation is not spontaneous but induced(3) and in which luteal function is essential at all stages of pregnancy(4), presents particularly favorable conditions for such a study. It is well known that ovulation in the rabbit occurs about 10 hours after the coital stimulus and that the changes leading up to ovulation are probably dependent on the release of LH into the circulation, though it is not known to what extent other gonadotrophic hormones are involved. Hypophysectomy later than 60 minutes(5) after coitus will not prevent ovulation, indicating that enough gonadotrophic hormone is released from the pituitary gland within a short period to ensure ovulation. There is a coincident depletion of anterior pituitary gonadotrophic material(6) and a rise in plasma gonadotrophic activity(7). Other experiments have indicated that about twice the necessary amount of ovulating hormone is present within the first hour after coitus(8). There is a degranulation of pituitary basophiles and the appearance within a few hours of characteristic modified acidophile or carminophile cells of pregnancy, tentatively identified as

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sites of luteotrophic hormone synthesis(9). The pituitary gland, however, is essential at all stages of pregnancy in the rabbit(10).

Plasma gonadotrophins have not been demonstrable in the estrous or pregnant rabbit unless very large amounts of blood are injected(11) in which case ovulation and formation of corpora lutea occur. The hypertrophy of the interstitial cells of the ovary in the pregnant rabbit(12) also suggests the continued action of a luteinizing or interstitial cell-stimulating hormone. Luteinizing hormone activity may now be measured in blood by the Parlow method (ovarian ascorbic acid depletion in pseudo-pregnant rats)(13). The method has satisfied stringent demands of sensitivity and precision (14). The only material so far identified which may introduce possible errors is vasopressin though the endogenous quantities of these compounds do not seem to be a significant problem in the assay(15).

Ovarian ascorbic acid depleting activity (OAADA) has been measured in the blood of estrous and in pregnant rabbit in the immediate postcoital period and on every day of pregnancy. The results are discussed.

Materials and methods. New Zealand White rabbits at all stages of pregnancy were used. Blood was obtained either by heart puncture under light sodium nembutal anesthesia or in the conscious animal through an indwelling polyethylene catheter in the common carotid artery. The catheter was brought out through the back of the animal's neck. Following each withdrawal of blood the tube was flushed with heparinized saline and sealed with heat. We are indebted to Dr. V. Popovic of Emory University for this method. About 10 ml of blood was removed for each assay. Heparin or EDTA (ethylenediaminetetraacetic acid, disodium salt) was used as an anticoagulant. Laparotomy was performed on all rabbits to identify ovulation sites in the early stages of pregnancy and to investigate the condition of the fetuses and placentas in the later stages.

The plasma from the rabbits was assayed in Holtzman rats received on the 25th day after birth. The rats were immediately divided into groups of 12 and injected subcutaneously with 75 International Units of pregnant mare's serum gonadotrophin (Progynon, Upjohn). Four days later they received a subcutaneous injection of 50 International Units of human chorionic gonadotrophin (Antuitrin S, Parke Davis). The rats were used on the 4th or 5th day after the last injection. It was essential that a full 4 days elapse from the last injection; otherwise the ovaries were very insensitive to depletion of ascorbic acid by the rabbit plasma. Ovaries weighing less than 70 mg were discarded.

The assays were carried out as follows: All injections were made slowly through a 27-gauge needle into the saphenous vein exposed under light ether anesthesia. Toxic reactions were encountered in some instances, especially after the injection of plasma from postcoital rabbits, but these were controlled by slow injection and by artificial respiration when needed. The following groups of animals were used in each assay:

1. A control group of 3 rats which received no injection; 2. a group of 3 rats which received 0.4 μg of luteinizing hormone (NIH · LH · S4) dissolved in 1 ml of saline; 3. a group of 3 rats which received 1.6 μg of LH in 1 ml of saline; 4. an experimental group of 3 rats which received 1 ml of rabbit plasma.

Two or three samples of rabbit plasma could be run simultaneously with the above controls. Plasma could be stored overnight in the deep-freeze; however, storage for a longer period resulted in loss of activity.

Both ovaries from each animal were then removed under ether anesthesia at the end of 1 or 4 hours after injection. The choice between these 2 times after injection was found by repeated experiment to be of no significance (see later), and the 4-hour period was finally adopted as a matter of convenience. In the first few assays performed a supply of LH was not available. In all later assays LH‡ was used at the above 2

‡ Luteinizing hormone (ovine; NIH • LH • S4) was made available to us by the Endocrinology Study Section, Nat. Inst. Health, to which grateful acknowledgment is made. The lyophilized material was diluted with lactose and stored at 4°C with no apparent loss of activity over a 2-year period.

dose levels. A line drawn through these 2 points showed a high degree of reproducibility with respect to degree of response and slope when tested repeatedly over a long period. The results reported here represent the ascorbic acid depleting activity of rabbit plasma at only one dose level (1 ml). Variation of individual readings for the 6 ovaries in each group rarely exceeded 5% above or below the mean. Results obtained without standards in the earlier assays were reexamined and coincided with those of later assays at all stages of pregnancy.

Both ovaries of control and experimental rats were removed under anesthesia, trimmed of fat and weighed on a torsion balance. They were then homogenized in fresh cold 2.5% metaphosphoric acid and filtered through Whatman #3 paper. The filtrates were usually stored overnight at 4°C with no apparent loss of activity. Ascorbic acid was measured by the method of Mindlin and Butler(16) using a Coleman Junior spectrophotometer.

Results were expressed as the percentage depletion of ascorbic acid in ovaries of rats injected with LH or rabbit plasma compared with the ascorbic acid content of the ovaries of control rats considered as 100%.

Eight estrous and 58 pregnant rabbits were used. Of the latter 27 were used for withdrawal of blood on one day in pregnancy, 2 were used on 2 separate days, 7 on 3 separate days, and 12 on 4 or more separate days. When multiple withdrawals were made on a single animal, the results were included only if the fetuses were shown at laparotomy to be in good condition following the last withdrawal of blood. In most cases, however, the rabbits delivered their young at term. Blood was withdrawn in almost all cases between 8 a.m. and 9 a.m.

Results of experiments to show the influence of the elapsed time following intravenous injection of LH on the percentage depletion of ascorbic acid are shown in Table I. No significant difference was observed.

Plasma levels of ovarian ascorbic acid depleting activity (OAADA) in estrous and pregnant animals (Fig. 1). The 8 estrous rabbits showed no significant OAADA in 1 ml of plasma. Plasma levels of OAADA were variably elevated throughout pregnancy. A study of the whole curve of plasma OAADA, for purposes of statistical analysis, suggested the division of pregnancy into 6 periods, as follows:

TABLE I. Depletion of Ovarian Ascorbic Acid at 1 and 4 Hours After Injection of LH.

μg LH injected in 1 ml saline	Depletion of ascorbic acid + 1 hr (%)	+ 4 hr (%)
.4	15	12
.8	27	28
1.6	40	41
2.0	44	40

(1) *Period 1 (0-3 days).* During this period, which includes the postcoital phase, plasma levels of OAADA were elevated (range, 15 to 30% depletion). The rise in OAADA began within 15 minutes following coitus.

(2) *Period 2 (4-5 days).* During this period plasma levels of OAADA were lower than in the first period (range, −5 to +12% depletion) being in 7 cases as low as in the estrous animals.

(3) *Period 3 (6-17 days).* This period corresponded with the last ⅔ of the period of pseudopregnancy in rabbits. It was marked by elevated plasma levels of OAADA (range, +10 to 35% depletion).

(4) *Period 4 (18-23 days).* Plasma levels of OAADA remained elevated above estrous levels during this period (range, +8 to +30% depletion).

(5) *Period 5 (24-30 days).* Plasma levels of OAADA declined in this period (range, −5 to +18% depletion), many of the animals showing levels similar to those of estrous rabbits. The end of this period corresponded approximately with the end of pregnancy (about 30 days).

(6) *Period 6 (31-33 days).* This period coincided with the end of pregnancy (about 30 days) and included the immediate post-parturitional period. Elevated levels of plasma OAADA in these animals which had delivered their young (indicated by half-moons in Fig. 1) suggest that there was a rise in plasma OAADA before, coincident with, or immediately following parturition.

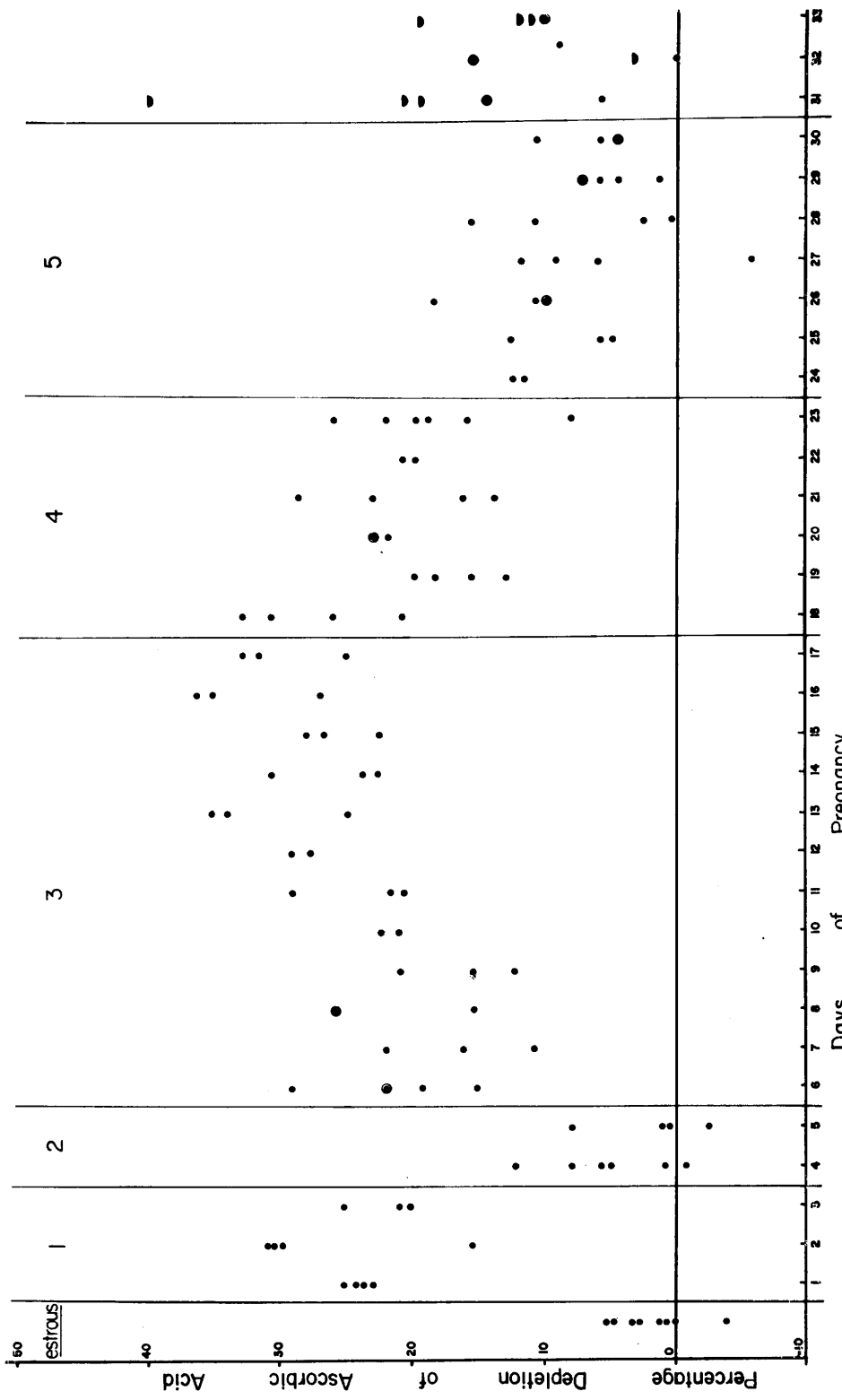

FIG. 1. Plasma levels of ovarian ascorbic acid depleting activity in estrous and pregnant rabbit. Each point represents mean depletion in 3 rats (6 ovaries). Larger points (●) are values for a single animal followed at intervals throughout pregnancy. Postparturient animals are indicated thus: ◑.

TABLE II. Mean Percentage Depletions of Ovarian Ascorbic Acid in Rats Injected with 1 ml of Plasma from Estrous Rabbits and Pregnant Rabbits at 5 Selected Periods of Pregnancy (See Fig. 1).

	Estrous	Period 1	2	3	4	5
		Days of gestation				
		1–3	4–5	6–17	18–23	24–30
N	7	11	10	37	20	23
$\bar{X}$	2.57	24.36	4.10	23.19	21.50	7.83
s	1.99	4.78	5.02	7.77	5.77	5.27

N = No. of estimations (not animals).
$\bar{X}$ = mean percentage depletion.
s = standard deviation.

One animal (indicated in Fig. 1 by a dot of greater size) was followed sequentially on the 6th, 8th, 20th, 29th, 31st and 32nd days after coitus. This animal delivered on the 33rd day. The levels of plasma OAADA in this animal throughout pregnancy were elevated in periods 3 and 4, fell in period 5 and rose in the 2 days preceding delivery (33 days).

A statistical analysis of the results in the first 5 of the 6 selected periods of pregnancy is presented in Table II.

The mean percentage depletions, numbers of animals and variation within each period are shown. To compare reliably the means in each group, the variances (s^2) were tested for homogeneity by Bartlett's X^2 test. When the estrous group was included, X^2 with 5 degrees of freedom was 15.61 with $p<0.01$ indicating heterogeneity. However, when the estrous period, in which the mean and the standard deviation were smaller than in any group was omitted, X^2, for the other periods was 7.62 with 4 degrees of freedom and with $p>0.10$ indicating homogeneity. Therefore, the means of the 5 groups during pregnancy were compared by the analysis of variance which showed highly significant differences among the means ($p<0.001$). Comparison within all pairs of means were then made by Duncan's Multiple Range Test(17). These results are summarized below:

Period 1	Period 3	Period 4	Period 5	Period 2
24.36	23.19	21.50	7.83	4.10

Any 2 means underlined by the same line do not differ significantly from each other and any 2 means not underlined by the same line do differ significantly from each other at $p = 0.01$.

Discussion. The ovarian ascorbic acid depletion assay of Parlow is now generally established as a valid and sensitive test for luteinizing hormonal activity. It is also effective for the measurement of gonadotrophic substances of placental origin such as human chorionic and pregnant mare's serum gonadotrophin which have some luteinizing activity. The application of the method to blood is not entirely satisfactory, however, and has so far limited the use of the assay as a clinical procedure(18). The ovarian ascorbic acid depleting activity (OAADA) described here in the plasma of pregnant rabbits may represent endogenous pituitary LH or LH-like material of placental origin, especially in the later stages of pregnancy. Fluctuations in the level of OAADA observed are: (1) an immediate postcoital rise lasting about 3 days, (2) a fall to zero or near-estrous levels on days 4 to 5, (3) a prolonged elevation from day 6 to about day 23, *i.e.*, throughout the major period of pregnancy, (4) a second fall to near-estrous levels from day 24 to day 30, and (5) a suggestive rise before parturition.

The postcoital rise in plasma OAADA correlates well with the expected release of pituitary LH into the blood, generally supposed to be a prerequisite for ovulation in the rabbit. However, actual measurements of plasma LH have not been made hitherto in this period, and the evidence for the postcoital release of LH is indirect and circumstantial.

The elevated plasma levels of OAADA throughout pregnancy, which will be assumed in the rest of the discussion to represent the activity of LH or LH-like material, must be considered in relation to the luteal and enhanced interstitial cell function characteristic of pregnancy in the rabbit. The observations of Marsh and Savard(1) that pituitary LH increases the *in vitro* production of progesterone by slices of human and bovine corpora lutea suggest that LH may be a physiological regulator of luteal function. Luteotrophic activity by ovine LH has recently been observed in the rabbit(19).

However, in the rat, LH has been considered to have a luteolytic role by which the life span of the corpus luteum is limited(2). It is certain that species differences are here of great importance, as in the case of prolactin which is said to be luteotrophic in the rat (20) and mouse(21) but which is neither luteotrophic nor luteolytic in the rabbit(19, 22). An elevated level of LH in the blood of rabbits during pregnancy is certainly inconsistent with a luteolytic role of LH in this animal. It is also noteworthy that the peak in the level of progesterone in the ovarian venous blood of rabbits occurs at the middle of pregnancy(23), thus coinciding with the highest levels of LH in the plasma as recorded here. Declining levels of plasma LH in the later stages of pregnancy coincide with a period of waning luteal activity(24). The suggestive rise in plasma LH activity just before parturition, clearly shown in one animal followed sequentially throughout pregnancy, may be correlated with the increasing dominance of estrogen over progesterone(25), with the onset of labor and with the characteristic postpartum estrous behavior of the rabbit.

Of particular interest is the apparent fall in the plasma OAADA on the 3rd and 4th days after coitus (period 2, Fig. 1). A possible explanation may be that this fall coincides with the depletion of pituitary stores of LH in which case the production and subsequent release of additional pituitary LH may not begin until the 5th day. It is significant that the first 2-4 days of pregnancy in the rabbit are associated with the structural organization and development of the corpora lutea. Though the corpora lutea develop normally for the first 36 hours after hypophysectomy, with some retardation of growth(5, 26) their functional maturation probably requires the continued action of LH. The role of LH in activation of progestin production by the ovarian interstitial cells before the onset of luteal function is of great potential importance and requires further investigation.

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