In the present work Vit. B_6 deficiency did not appear to affect the percentage of administered C^{14} which appeared in the urine as oxalate. This may have been a reflection of the often reported defect in the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid and alanine in Vit. B_6-deficient rats. Thus, although the Vit. B_6-deficient rats were synthesizing more oxalate than their controls, less labelled precursor was available to them with the result that the specific activity of the oxalate excreted by the Vit. B_6-deficient rats was less than that of their controls.

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Gestation in Salpingectomized-Ovariectomized Progesterone-Treated Rabbits.* (28745)

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Pincus and Kirsch(1) ligated one uterine tube at the isthmus in rabbit at 17 hours after fertile mating; 6 days later, degenerated embryos at the late morula or early blastocyst stages were recovered from the ligated tubes. The embryos on the non-ligated side had entered the uterus and developed normally. Similar results were obtained when the interval between mating and ligation was some 32 hours(2). These results suggested that the uterine tubes were essential to the expansion of blastocyst. It is well known that the fertilized egg does not expand during its 72-hour journey in the uterine tube. At 3 days *post coitum* the utero-tubal junction relaxes, allowing the flow of the luminal fluid of the uterine fluid containing the late morulae and early blastocysts(3). The complex processes of active transport associated with the expansion of the blastocysts are not known.

Ovariectomy does not interfere with the passage of embryos through the uterine tube of the rabbit(2,4,5), rat(6), cat(7) and guinea-pig(8). However, ovariectomy causes termination of pregnancy in the early stages

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of several mammalian species, *e.g.*, rabbit, rat, cat, opposum and ground squirrel(9,10). In these species, it is not clear whether ovariectomy causes the degeneration of the preimplantation blastocyst or inhibition of blastocyst implantation.

Exogenous progesterone with or without estrogen enables implantation to take place normally in ovariectomized mammals. Westman(11) reported that ovariectomy in the rabbit caused degeneration of the embryos before the late morula stage. Following destruction of the corpora lutea by thermocautery, King(12) observed a relatively high incidence of retarded and degenerated rabbit embryos 41 to 96 hours after mating, but some early blastocysts were obtained.

The purpose of this study was to examine the effects of removing uterine tubes, 3½ days *post coitum,* on expansion and implantation of blastocyst, and the effect on normal gestation and parturition in ovariectomized progesterone-treated rabbits.

Materials and methods. Sixty-six nulliparous New Zealand large does were divided at random into 6 groups. The does were bred twice to fertile bucks and injected intravenously with 15 i.u. of HCG (Upjohn). The experimental treatments were as follows:

Group I: At 3½ days *post coitum* the does were ovariectomized and salpingectomized biliterally, injected with 4 mg progesterone daily and autopsied 6 days *post coitum.*

Group II: At 3½ days *post coitum* the does were ovariectomized bilaterally, salpingectomized unilaterally, injected with 4 mg progesterone daily and autopsied at 6 days *post coitum.*

Group III: At 2 days *post coitum* the does were ovariectomized bilaterally, injected with 4 mg progesterone daily and autopsied 6 days *post coitum.*

Group IV: Intact does were sham-operated 2 days *post coitum* and injected with 4 mg progesterone daily starting 2 days *post coitum* and autopsied 6 days *post coitum.*

Group V: Intact does were autopsied 6 days *post coitum.*

Group VI: The does were salpingectomized and ovariectomized bilaterally at 3½ days *post coitum,* laparotomized on day 9 *post coitum* to count the number of implantation points. This group was allowed to term and nests were provided at 30 days *post coitum.*

In Groups I through V, the does were autopsied, the uterine horns were flushed with physiological saline, and the flushings examined microscopically (× 45) to determine the abnormality of blastocysts. The outer diameter of blastocysts was measured using an ocular micrometer (× 37.5). The blastocysts were fixed in absolute methanol for 30 minutes and the diameter of embryonic disc was measured. The number of corpora lutea was counted at the time of ovariectomy or autopsy and weight of the 2 uterine horns was recorded.

Results and discussion. In Groups I to IV the average number of corpora lutea per group varied from 9.7 to 12.1. Viable blastocysts in both horns as a percentage of total number of corpora lutea varied from 52 to 90. The percentage of viable blastocysts per group expressed to total number of recovered blastocysts varied from 89 to 100. Ovariectomy with or without salpingectomy reduced the percentage of viable blastocysts expressed to number of corpora lutea by 10 to 20% (Table I). This may be due to a delay in the transport of embryos through the uterotubal junction as a result of traumatization during ovariectomy. However, the animals that underwent a sham operation did not show a reduction in percentage of viable blastocysts. Viability of blastocysts was not affected by ovariectomy with or without salpingectomy as percentage of blastocysts recovered did not vary between the experimental groups.

The mean outer diameter of blastocysts per group varied from 2.86 to 3.36 mm. The mean diameter of the embryonic disc per group varied from 0.80 to 1.03 mm. Analysis of variance showed that there was no significant difference between groups in the outer diameter of blastocyst and in the diameter of embryonic disc. There was great variability within group and within the same litter in the size of blastocysts. The nature and significance of such variability is still unknown and it does not seem that the size

TABLE I. Diameter of Blastocysts and Embryonic Disc 144 Hr *Post Coitum* in Salpingectomized-Ovariectomized Progesterone-Treated Rabbits.

Group	Treatment: Surgical	Treatment: Progesterone daily (mg)	No. of corpora lutea: Range	No. of corpora lutea: Mean	Wt of both uterine horns (g): Range*	Wt of both uterine horns (g): Mean†	Total No. of blastocysts (viable + degenerating)	% of viable blastocysts‖: A	% of viable blastocysts‖: B	Diameter of embryonic disc (mm): Range	Diameter of embryonic disc (mm): Mean	Outer diameter of blastocysts (mm): Range	Outer diameter of blastocysts (mm): Mean
I	Salpingectomized + ovariectomized (both bilateral)	4	6-13	10.0	8.61-12.57	10.71	59 + 0	74	100	.65-1.27	.80	1.91-3.93	2.88
II	Salpingectomized (unilateral) + ovariectomized (bilateral)	4	9-17	12.1	4.25- 7.15	11.11	‡ 33 + 3 § 46 + 2	63 67	92 96	.51-1.64 .48-1.39	.94 .94	1.00-4.25 .48-4.27	3.01 2.89
III	Ovariectomized (bilateral)	4	7-14	10.3	6.90-12.20	8.82	70 + 9	52	89	.41-1.36	.97	1.49-4.00	2.86
IV	Intact (sham operation)	4	7-13	10.4	6.28-12.69	9.94	63 + 2	86	97	.63-1.40	.90	1.32-4.94	3.17
V	Intact	Nil	3-14	9.7	3.60-12.50	10.08	113 + 4	90	97	.35-1.57	1.03	1.33-4.27	3.36

* Figures represent side rounded off to nearest value; then the 2 sides averaged.
† Averages of combined sides divided by number of does where horns were weighed.
‡ Blastocysts recovered from side where uterine tube was removed.
§ Blastocysts recovered from side with intact uterine tube.
‖ A = Total No. of viable blastocysts in both horns expressed to total No. of corpora lutea in ovaries.
B = Total No. of viable blastocysts in both horns expressed to total No. of blastocysts recovered in ovaries.

of blastocysts is related to the number of corpora lutea or to the weight of uterus.

In salpingectomized-ovariectomized does, examined at 9 days, implantations in both horns ranged from 4 to 13, and 78% of the number of corpora lutea was represented by implantations. The fetuses survived to term; a few of the young were stillborn. In some does parturition did not take place until 33 days *post coitum* and the animals when autopsied had viable fetuses. The observed abnormal parturition and lack of normal nesting behavior was probably due to faulty endocrine environment (lack of estrogen) rather than absence of the ovaries or the uterine tubes.

It would appear from the results that salpingectomy had no effect on the expansion of the blastocysts and their survival, implantation and normal gestation. Normal gestation in the rabbit could be maintained, after ovariectomy and salpingectomy, by injection of exogenous progesterone. There is no evidence to suggest that the presence of uterine tubes or the luminal tubal fluid is necessary for normal reproduction in the rabbit. It is essential to distinguish between the effect of tubal ligation on embryos still in the uterine tubes and the effects on the embryos which passed to the uterine horns.

Summary. Nulliparous rabbits were bred, ovariectomized and salpingectomized bilaterally at 3½ days *post coitum*, injected daily with 4 mg of progesterone and autopsied at 6 days *post coitum*. The blastocysts were recovered, examined for their normality and their diameters measured. Some salpingectomized-ovariectomized animals that were allowed to term were laparotomized at 9 days *post coitum*, to count and measure implantation swellings. Control groups were subjected to sham operations. The percentage of viable blastocysts to the total number of corpora lutea varied from 52 to 90. The percentage of viable blastocysts varied from 89 to 100. The viability of blastocysts was not affected by ovariectomy with or without salpingectomy. Ovariectomy with or without salpingectomy reduced the percentage of viable blastocysts expressed to number of corpora lutea.

Groups did not differ significantly in the outer diameter of blastocysts and in the diameter of embryonic disc. There was great variability within group and within the same litter in the size of blastocysts and their survival. Implantation and normal gestation in the rabbit could be maintained, after ovariectomy and salpingectomy, by injection of exogenous progesterone.

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Effect of Alloxan on Malic Dehydrogenase Activity of Microdissected Mammalian Pancreatic Islets.* (28746)

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Dunn *et al*(1) first reported that alloxan injection caused a selective necrosis of the pancreatic beta cells, with consequent induction of diabetes. It had been suggested that alloxan directly or indirectly inactivates a critical sulphydryl enzyme(s) within the beta cell(2). More recent studies, however, indicate that alloxan may kill the beta cells by altering the permeability of the cell membrane. Tracer doses of alloxan apparently do not enter the beta cell(3). However, concentrations of alloxan equivalent to the diabetogenic dose, increased the *in vitro* permeability of fish islet tissue to mannitol, a substance which is normally restricted to the extra cellular space(4).

Hitherto, it had not been possible to study directly the changes in the mammalian islet tissue following alloxan administration. The techniques developed by Lowry and coworkers(5) permit microdissecting, weighing and enzymatic analysis of submicrogram quantities of tissue. Using these techniques, we have undertaken a systematic study of the changes in the enzyme activity in the rat islet tissue following administration of diabetogenic agents.

We are reporting here the changes observed in malic deyhdrogenase (MDH) activity of the microdissected islets following alloxan administration.

Materials and methods. The male rats used were litter mates, 2 to 3 months of age, bred in this laboratory. All animals were fasted overnight, and blood samples were taken from the tail vein for glucose determination(6). Experimental animals were given a single intravenous injection of alloxan (40 mg/kg body weight(7)), whereas controls were injected with distilled water. One animal from each litter was always used as a control. The litter mates were decapitated (by guillotine) at various intervals (24, 48, 72 and 168 hours) after alloxan injection. In another series of experiments, litter mates were sacrificed at 12 and 18 hours after alloxan. The pancreas was removed, and quickly frozen in liquid nitrogen (−196°C). As a rule, the blood sugar level at time of sacrifice was over 300 mg/100 ml. The frozen tissue was sectioned at 20 μ in a

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