insoluble aggregates. This reaction is one of loose combination, since it can be reversed by readjusting the pH to 7.0. Non-specific capsular swelling of pneumococci can be produced also under these conditions, but, in addition, the protein-pneumococcus mixture at pH 4.0 must be relatively salt-free. It is felt that an altered state of the capsular gel is produced by the interaction of protein and carbohydrate. In the absence of ionized salts, the polysaccharide-protein gel becomes increasingly hydrophilic and therefore capable of enlarging to produce the "quellung" reaction. Both specific and the described acid-protein quellung can be produced in the absence of calcium in distinction to Lofstrom's non-specific "quellung" phenomenon.

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Effect of Alloxan Diabetes on Reproduction in the Rat.*

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The administration of alloxan to experimental animals will produce a condition analogous to diabetes mellitus in man.[1,2,3] The principal effect of the drug is a selective necrosis of the islets of Langerhans in the pancreas, although there is slight damage to other tissues.[4,5] The experimental diabetes may be transitory in character and the animal recover, or it may be permanent, becoming progressively more severe until the animal succumbs. The dose of the alloxan administered is the most important factor in the ultimate course of the diabetic state.

Friedgood and Miller[6] injected alloxan 4 days before parturition and obtained normal litters. This observation suggests that terminal diabetes is not sufficient to interfere with the development of fetuses. The high incidence of pregnancy complications in human diabetics prompted us to restudy the problem and the following experiments were designed to provide additional pertinent information.

Methods. The objectives of these experiments were three-fold: (1) to determine the effect of alloxan diabetes on cyclical activity and fertility in the rat, (2) to study reproduction in rats made diabetic early in pregnancy and (3) to evaluate the course of pregnancy and labor in the rat with diabetes controlled adequately by insulin.

Young mature female rats, weighing 175 to 250 g, of the inbred Sprague-Dawley stock raised and maintained in our laboratory were used throughout these studies. They were given free access to a balanced diet which was supplemented by milk as well as wheat germ oil once a week. Sex cycles were followed routinely employing the standard vaginal

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smear technique. The animals were mated by introducing them to males of known fertility and the onset of gestation noted by the presence of spermatozoa in the vaginal smear. It was further checked by the appearance of the placental sign on the twelfth day of the gestation.

Fasting blood sugar levels on venous blood from the tail were determined at 2- or 3-day intervals throughout these experiments by Somogyi's micro-modification of the Schaffer and Hartmann method. The animals were placed in metabolism cages during the day at least 4 hours prior to the blood sugar determinations in order to insure fasting levels. In those animals receiving insulin for the control of the hyperglycemia, qualitative sugar determinations on the urine at frequent intervals made it possible to follow the course of the diabetes.

Cyclical activity was studied in the first group of 6 animals. After a baseline of normal estrous cycles was established for each animal, it was injected with alloxan intravenously. The vaginal smears were followed for a sufficiently long period so that the character of the diabetes induced in the animal could be established and the effect of the hyperglycemia on estrous cycles studied. Varying amounts of alloxan were used. Some of these animals were subsequently mated and their pregnancies followed.

A second group of 49 normal female rats was placed with males and as soon as pregnancy was diagnosed by the appearance of spermatozoa in the vaginal smear they were segregated. Prior to the fifth day of the pregnancy they were injected with varying amounts of alloxan. Blood sugar determinations were made on alternate days throughout their pregnancies. Three of these animals were followed through a second pregnancy. The animals were carefully observed for the accidents of pregnancy.

A third group of 7 rats was made diabetic by alloxan. When it was certain that the diabetes was permanent in character, the hyperglycemia was controlled by insulin. These animals were allowed to mate and the resultant pregnancies carefully studied.

Results. The Effect of Alloxan Diabetes on the Estrous Cycle. After the normal estrous cycle had been established in 6 rats, each received 40 mg of alloxan per kilo of body weight. One animal died 3 days after the injection at which time the fasting blood sugar value was 379 mg %. Two of the animals developed a transient diabetes only and in these the blood sugar levels returned to the normal by the eighth day. The hyperglycemia in these animals reached their highest values, 160 and 207 mg %, on the fifth day following the administration of alloxan. The remaining 3 animals developed permanent alloxan diabetes. Fasting blood sugar levels ranged from 288 to 360 mg % throughout the period of observation.

Following the development of hyperglycemia the estrous pattern changed. Estrous smears occurred with considerable irregularity and the interval was prolonged. The normal 4 or 5 day pattern was replaced by one of 9 to 12 days, the animal remaining in the diestrous condition the greater part of each cycle.

Effect of Alloxan on Mating. Nine diabetic animals in whom blood sugar levels vary from 225 to 350 mg % were placed with males of known fertility. When estrus occurred these animals mated normally as evidenced by the presence of spermatozoa in the vaginal smear. However, the irregularity of the estrous cycles and the prolonged interval between estrous periods lengthened the time necessary for mating.

Three of the animals died 4 or 5 days after spermatozoa were found in the vagina. These animals had normal gestations and their deaths were probably due to the diabetes. Two rats who had hyperglycemia prior to pregnancy developed normal blood sugar levels during the gestation. These 2 animals carried their pregnancies to term and delivered normal litters uneventfully. The remaining 4 animals continued to exhibit a marked hyperglycemia with levels ranging from 210 to 360 mg %. The placental sign appeared on the twelfth day of the gestation. However, at the end of their pregnancies they delivered macerated placentas and no fetuses.

TABLE I.
Summary of Diabetic Rats Treated with Protamine Zinc Insulin. Note the lack of difference in size of litters or weights of fetuses between normal and diabetic animals

Rat No.	Units of Protamine zinc insulin/day	No. of fetuses in litter	Sex M	Sex F	Avg wt, g M	Avg wt, g F	Blood sugar P.P. No insulin mg %	Remarks
372	0.75	9	4	5	6.2	4.5	215	F.B.S. 2 days postpartum
470	0.75	5	2	3	5.0	4.0	373	" " "
471	0.75	11	4	7	6.2	5.0	360	" " "
580	0.75	4	2	2	5.5	6.0	270	" " "
269	0.75	5	2	3	6.5	6.0	399	" " "
183	0	9	4	5	6.2	6.2	Normal	Breeding stock
112	0	6	4	2	5.2	5.0	"	" "
92	0	10	4	6	5.0	5.0	"	" "
100	0	5	2	3	6.7	5.6	"	" "
186	0	4	2	2	5.6	5.6	"	" "

Effect of Alloxan on Pregnancy. A total of 49 pregnant rats were injected intravenously with alloxan, the amount ranging from 30 to 60 mg per kilo, prior to the fifth day of gestation. The most satisfactory dose from the viewpoint of developing a permanent diabetes was 40 mg per kilo. Twenty-seven of these animals died at various stages of their pregnancies and they were studied postmortem. Fourteen of the animals were sacrificed at various periods in their pregnancies in order to provide fresh material for histologic study. Five of the animals survived their pregnancies and delivered macerated placentas at term. The remaining 2 animals did not maintain their hyperglycemia during the pregnancy and they were sacrificed on the ninth and fourteenth days of the gestation. Their pregnancies were progressing uneventfully.

Gross and histologic examinations of the reproductive organs of the diabetic rats revealed typical changes in the entire group. The pregnancies progressed normally until the twelfth day of the gestation. The fetuses and the placentas were normal. Following this period the fetuses died and were slowly absorbed. Necrotic fetuses in various stages of degeneration and absorption were present in the animals sacrificed at varying periods of pregnancy. (Fig. 1.) The placentas continued to grow after fetal death and remained attached to the uterine wall until separated during parturition. The size of the placenta at term was distinctly smaller than the normal placenta probably because of the absence of the fetus. The placentas were delivered at term after an uneventful parturition and all visible remnants of the fetus had disappeared.

These experiments provided proof that the rat made permanently diabetic by the administration of alloxan very early in pregnancy could not deliver a living litter at term because of fetal death prior to the twelfth day. The next step was to see if the control of the hyperglycemia by insulin would result in normal pregnancies. A group of 7 rats was made diabetic by the intravenous injection of 40 mg of alloxan per kilo of body weight. Their fasting blood sugar levels ranged from 253 to 458 mg %. Two weeks later when it was made certain that the diabetes was permanent the animals were placed on protamine zinc insulin, the amount administered being controlled by daily qualitative urine sugar determinations. When the diabetes was controlled by the insulin, the animals were allowed to mate and become pregnant.

The most striking result following the control of the diabetes by insulin was the resumption of normal, regular estrous cycles. The animals became pregnant, carried through to term uneventfully and delivered normal living litters. Two of these animals died at term and subsequent autopsy examinations did not reveal the cause for the deaths. The placentas and the young were normal.

Insulin therapy was discontinued several days after delivery in order to determine the status of the diabetic state. In all instances blood sugar values returned to their previous levels at which they continued as long as

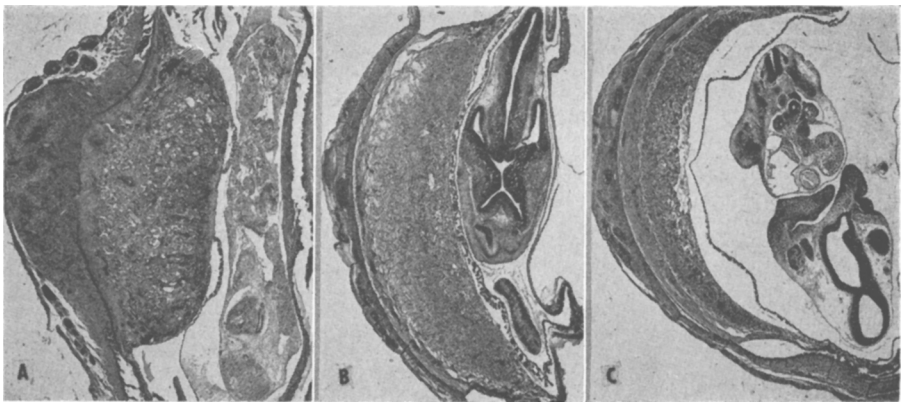

FIG. 1.
Photomicrographs through gravid uteri. A. Permanently diabetic rat at 16 days of gestation. Note the necrotic condition of the fetus. B. Rat with transient diabetes at 15 days gestation. The diabetes lasted 7 days. The fetal structures appear normal. C. Control animal at 14 days gestation.

no insulin was administered. The pregnancies and the insulin therapy did not alter the diabetes. (Table I).

Discussion. Alloxan diabetes in the rat alters the normal cycles, decreases fertility because of this change and affects pregnancy seriously. It is important to make certain that the permanent diabetic state has been induced. Transitory hyperglycemia is a frequent result following an inadequate amount of alloxan. Some of the animals used in these experiments recovered from their diabetic state early in pregnancy and subsequently delivered normal litters at term. Fasting blood sugar levels vary considerably and in the animals used in this study they ranged from 186 to 425 mg %.

Living litters were delivered in none of these diabetic animals. Characteristically, the pregnancy progressed normally until the midpoint of the gestation when the fetus succumbed, following which it was slowly absorbed so that it was absent at term. The placentas remained attached and were delivered at term following a fairly normal parturition.

These observations suggest that terminal diabetes is not sufficient to interfere with the development of the fetuses and that the critical period for the fetus was midpregnancy.

Is it the hyperglycemia that interferes with the estrous cycles and normal gestation in the rat? If the effect of insulin is simply the control of the hyperglycemia, this may be so. A group of diabetic rats were adequately controlled by insulin following which the estrous cycles returned to normal, they conceived and delivered normal living litters at term. The weight of the individual fetus and the gross weight of the litter compared favorably with normal control animals in the colony. It is possible that insulin does more than neutralize excessive blood sugar and diabetes represents more than an altered carbohydrate metabolism.

Summary and Conclusions. The effects of alloxan diabetes on ovarian activity and pregnancy were studied in a series of 63 adult female rats. The development of permanent hyperglycemia resulted in an alteration of the normal estrous pattern, so that the intervals were greatly prolonged. Pregnancy progressed normally until about the twelfth day following which the fetus died and was slowly absorbed. The placentas were retained and were delivered on the day of parturition. The adequate treatment of the alloxan diabetes by insulin resulted in normal pregnancies and live litters delivered at term.