chemicals do reach and affect eggs, spermatocytes and mature sperm, and some support for this has been presented, the negative results obtained with these technics may be due to the relatively short duration of the treatments or to differences in the mutability of the germ plasm at various stages of its differentiation.

Summary. Vaginal douches of 4% methyl bis (β-chloroethyl) amine hydrochloride produced 53 lethals in 5488 egg and sperm X chromosomes tested while only 13 lethals were observed in the 6271 sperm or egg X chromosomes tested after treatments with formaldehyde, copper sulfate, 2,4-dinitrophenol, hydrazine hydrate and trypsin. The practicality of the vaginal douche technic for the induction of mutations with N mustard in both sperm and eggs, suggested from previous experiments, is confirmed in that 30 lethals occurred in the 2951 sperm tested and 23 in the 2537 eggs tested. The number of sperm X chromosome lethals seems to be considerably higher in F_1 females resulting from single copulations within a two hour period from the time of injection (23 lethals in 1416 tests) than it is in females from copulations after this interval (7 lethals in 1535 tests); the mutation rate for the egg X chromosome under these conditions remains apparently unaffected (9/1083 and 14/1454, respectively). Whereas the 30 sperm lethals seem to be distributed at random among the F_1 females oviposited at various times, the frequency of egg lethals in individuals oviposited during the first two days (18/649) is statistically larger than the frequency of lethals in F_1 females coming from eggs deposited after that time (5/1888).

The use and effects of vaginal douche treatments are discussed and results of the experiments evaluated in the light of studies of other workers testing these chemicals in Drosophila.

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Effect of Alloxan Diabetes on Fertility and Gestation in the Rat.*

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The discovery of the diabetogenic properties of alloxan[1] gave to the study of metabolism a tool by means of which many vexing problems may be investigated. One of these is the effect of diabetes on reproduction, a problem whose importance has been emphasized by White and Hunt[2] in extensive investigations on human diabetics. Reproduction in animals made diabetic with alloxan has been studied by Miller,[3] Friedgood and Miller,[4] Davis, Fugo and Lawrence[5] and Hultquist.[6] Reported below are some observations on the rat made diabetic with alloxan in which the problem of fertility, gestation and the via-

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1 Dunn, J. S., and McLetchie, N. G. B., *Lancet*, 1943, **2**, 384.

2 White, P., and Hunt, H., *J. Clin. Endocrinology*, 1943, **3**, 500.

3 Miller, H. C., *Endocrinology*, 1947, **40**, 251.

4 Friedgood, C. E., and Miller, A. A., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 61.

5 Davis, M. E., Fugo, N. W., and Lawrence, K. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 638.

6 Hultquist, G., *Acta Path. et Microbiol. Scand.*, 1948, **25**, 131.

TABLE I.
Reproduction Record of Untreated Diabetic Animals.

Rat No.	Blood sugar, mg per 100 ml: Before breeding	During gestation	Post-partum	Gestation period (days)	No. of offspring: Living	Dead	Avg body wt of offspring, g
18	398	366		22	8		5.2
29	354	328	422	23	9		5.3
30	460	396	468	23	8	2	4.8
48	372	236	452	23	9	1	5.7

TABLE II.
Reproduction Record of Insulin-Treated Diabetic Rats.

Rat No.	Blood sugar: Before insulin	With insulin	Daily insulin units	Gestation period (days)	No. of offspring per litter: Living	Dead	Avg body wt of offspring, g
24	549	280	7	22	7		4.93
29	422	236	9	23	10		5.50
30	468	228	9	22	6	1	4.79
56	474	186	7	23	8		4.75
62	476	137	9	24	4	1	5.07

bility of the fetus were investigated.

Experimental. Young female white rats, 90 to 120 days of age, from our own colony were used. The animals were kept in individual cages and were fed Purina Laboratory Chow. Lettuce was given once a week. Alloxan was administered intravenously via a tail vein in the amount of 55 mg per kilo of body weight. The method was essentially that of Lazarow and Palay.[7] The success of the production of diabetes was ascertained by the determination of the sugar content of blood obtained from the tip of the tail. A micro-modification of the method of Folin and Wu[8] was used for this determination.

The estrous cycle was followed by the vaginal smear method. When the animals were found to be in estrus, they were placed overnight with a young male of known fertility. On the following day, which was counted as the first day of gestation, the female was isolated. When the young were born they were counted and weighed within approximately 8 hours (maximum time). In all groups they were left with the mother so that her success in rearing the young might be observed.

Four groups of animals were studied. The first group received no treatment of any kind and served to establish the reproductive pattern of this strain of rats. Alloxan diabetes was produced in the animals of the second group, after which their ability to reproduce was studied without further treatment. The diabetic animals of the third group were given regular insulin twice daily with frequent determinations of the blood sugar until the estrous cycle returned. The time required for the resumption of estrus varied from 9 to 40 days. They were placed with the male at that time. The fourth group consisted of those animals which began pregnancy as diabetics and which demonstrated subsidence of hyperglycemia during the course of gestation.

Results. Normal Control Group. Fifteen rats were used as normal controls. The estrous cycle was 4 to 5 days in length. Fourteen had successful pregnancies, but 3 had to be placed with the male twice, 1 three times and 1 six times before they conceived. The remainder conceived at the first mating. The length of the gestation period was 22 to 23 days. The litters ranged from 4 to 13 in number and

[7] Lazarow, A., and Palay, S. L., *J. Lab. and Clin. Med.*, 1946, **31**, 1004.

[8] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

the weight of the newborn animals (average weight of the young in each litter) varied from 4.65 to 6.6 g.

Untreated Diabetic Group. The animals in this group were treated with alloxan and the vaginal smears were followed daily thereafter. Estrus appeared at intervals of 3 to 8 days following the alloxan administration. There were 13 diabetic animals in this group of which 9 failed to reproduce. The placental sign was observed in 3 of the latter 9 rats and a vaginal plug was seen in 4 of the 9 after they were with the males. In these animals the blood sugar values ranged between 365 and 468 mg per 100 ml.

The data concerning the 4 rats which did bear litters are given in Table I. Rat 18 killed and ate her young soon after parturition. The young from rat 30 died on the fourth day after parturition. Rat 29 cared for her young, but on the 14th postpartum day it became evident that she was unable to nourish them adequately and they were placed with a foster mother. They quickly gained weight and were successfully reared to the weaning age. Rat 48 reared all of her 9 live young to the weaning age.

Insulin Treated Diabetic Group. These animals were treated with regular insulin until a definite decrease in blood sugar had been established. With adequate treatment the estrous cycle was resumed in 5 of 7 animals even though the blood sugar values had not reached a normal level. The results of the reproduction experiment with this group are recorded in Table II. Of the 5 which resumed estrus, every one conceived and bore a viable litter. The young of rat 24 lived only 2 days. Rat 62 bore 4 live and 1 dead offspring, and the 4 live ones died within 3 days.

Rats 29 and 30 had borne litters while diabetic and untreated (Table I). Their estrous cycles were not resumed after the birth of their young until insulin treatment was begun. They then conceived and bore litters. Rat 29 bore 10 live rats which were reared to the weaning age and 6 living and 1 dead young were born to rat 30. The living young died within 3 days.

Rat 56 bore 8 living offspring which were accidentally sacrificed soon after birth.

Untreated Group with Transitory Diabetes. One group of 5 animals had only mild or transitory diabetes. Indeed, in 2 of these animals the blood sugar never did reach a level which would justifiably place them in the diabetic group. However, they were bred and produced litters, and the blood sugar decreased during the course of pregnancy to normal levels. The number of rats per litter varied from 5 to 13 and the average weight of the young varied between 4.6 and 6.1 g. The litter of one of these rats was born dead. These results seem to indicate that the administration of alloxan does not interfere with the ability to reproduce when it has been unsuccessful in the establishment of diabetes.

Discussion. The most recent report on this problem is that of Hultquist.[6] He produced diabetes both by pancreatectomy and by alloxan administration. Most of his animals were made diabetic after pregnancy was established and "only in a few instances" did he observe pregnancy in an already diabetic animal. He also observed that insulin improved the chances of the mother to bear young and that only one of many animals completed gestation without insulin treatment. The results herein reported show a higher percentage of successful pregnancies in untreated diabetic animals than his report seems to indicate, but also, marked improvement was observed when insulin was given, even though, again in agreement with Hultquist, the blood sugar was not regulated to non-diabetic values.

The experiments of Davis, Fugo and Lawrence[5] vary somewhat from the present ones. They observed a prolongation of the sex cycle from 4 to 5 days to 9 to 12 days with irregularity, in contrast to the observations reported above which showed one, or at the most, 2 estrous periods following a diabetogenic dose of alloxan, after which the cycle was not restored unless insulin was administered. Furthermore, none of their diabetic animals which were bred after becoming diabetic produced litters, in contrast to the 4 among 13 rats which accomplished successful gestation in the present experiments. The

smears were not examined for spermatozoa in our untreated diabetic rats after they had been with the male, and it is possible that some of the 9 animals which did not conceive failed of successful coitus.

When diabetic animals were treated with insulin their cycles returned and they were successfully bred and produced litters, in agreement with the findings of Davis, Fugo and Lawrence. The young either were not in good health or the mother's milk supply was inadequate as evidenced by the early death of all but one of the litters. Also confirming the findings of these authors, we observed that those animals which recovered spontaneously from hyperglycemia were able to reproduce. Alloxan, *per se*, in the doses used did not interfere with the ability of these animals to reproduce.

Miller[3] reported a successful gestation in 3 diabetic rats which received no insulin. They were only mildly diabetic as judged by the mild glycosuria. Friedgood and Miller[4] gave alloxan to pregnant rats on the fourteenth day of gestation. They determined that the blood of the fetuses was hyperglycemic at a level only slightly below that of the mother, and that the blood sugar of the offspring had returned to normal within one day. In the present experiments it was observed that the young of the 2 surviving litters from diabetic mothers had blood sugar values within the normal range. These determinations were performed after the young had been weaned. In consideration of these findings and the report of Dohan and Lukens[9] that short exposure of cats to hyperglycemia will produce diabetes, the resistance of the fetus of the rat to maternal hyperglycemia needs further investigation.

Abnormally large body weight is commonly observed in babies borne by human diabetics.[10] The young rats in this experiment, both from treated and untreated diabetic mothers did not exceed in weight those from non-diabetic mothers. In fact, the highest average weight in the litters occurred in the offspring of one of the mothers of the non-diabetic group.

The existence of diabetes in the mother, whether treated or untreated, did not affect the length of the gestation period significantly.

Summary. Rats were made diabetic with alloxan and their ability to reproduce was studied under various conditions. The following observations were made: (1) the estrous cycle ceased in diabetic females and was restored by the administration of insulin; (2) 4 of 13 untreated diabetic animals, which were bred before the estrous cycle ceased, produced litters; (3) insulin treated diabetic animals reproduced in spite of a continuing hyperglycemia; (4) transient hyperglycemia did not interfere with reproduction and (5) diabetes in the mother did not result in hyperglycemia in the offspring in the few animals tested.

[9] Dohan, F. C., and Lukens, F. W. D., *Endocrinology*, 1948, **42**, 244.

[10] Joslin, E. P., Root, H. F., White, P., Marble, A., and Bailey, C. C., The Treatment of Diabetes Mellitus, Lea and Febiger, Philadelphia, 1946, 773.