in the propylene glycol series. Complete loss of viability of all chlamydospores after 48 hours' exposure was established at a prodigiosin dilution of 1:100,000.

Summary. Prodigiosin was fungistatic for *Coccidioides immitis* in dilutions through 1:500,000. It was fungicidal after 48 hours' exposure in dilutions through 1:100,000. Prodigiosin hydrochloride, perchlorate and a water soluble glutamic acid derivative demonstrated similar *in vitro* effectiveness.

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Pregnancy in Alloxan Diabetic Rats. (17534)

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Because of the well recognized frequency of complications in the course of pregnancy in clinical diabetes, this study was undertaken to determine the course of pregnancy in rats suffering from uncontrolled chronic diabetes. Since August 1946, when these studies were begun, 4 reports(1-4) have appeared dealing with this subject each with a slightly different approach to the problem and each with somewhat varying results.

Methods. Twenty-seven 70-90 day old Wistar strain female rats were fasted overnight and given a single subcutaneous dose of alloxan varying from 140-175 mg per kg. Where a single dose failed to produce a persistent diabetic state, repeated injections of increasing doses were employed up to 205 mg per kg. In some rats it was necessary to administer as many as nine doses of alloxan in order to achieve permanent diabetes. In 2 rats permanent diabetes did not appear despite repeated injections of alloxan. During the entire period covered by these studies the animals were kept in individual metabolism cages and the following data were obtained daily, 7 days a week, for periods up to 448 days: (a) total fluid intake, (b) total food intake, (c) total urine volume, (d) total quantitative glycosuria, (e) qualitative acetonuria, (f) vaginal smears for determination of oestrus. The animals were given free access to a constant mixture of crushed Purina Lab Chow (Protein 25%, Carbohydrate 50%, Fat 6.3%) reinforced with the following: reduced iron 0.2 g; sodium chloride (iodine-free) 10 g; sodium chloride (iodized) 2.7 g; riboflavin 5 mg, vitamin B_6 5 mg, vitamin D 3000 U.S.P. units, vitamin E 100 mg, choline 15 mg, per kilo of chow in order to bring the ration up to an adequate level for breeding as recommended by the Ralston Purina Company.

Because of the frequent occurrence of a transient diabetic state after alloxan injection, matings were performed when possible only after the establishment of permanent diabetes by placing one young adult male of the same strain into the metabolism cage with each female on the day of oestrus. Animals were considered definitely diabetic only when they fulfilled both of the following criteria: (1) persistent glycosuria greater than 0.5 g per 24 hours as measured by Benedict's Picrate method, and (2) repeated non-fasting venous blood sugars in excess of 200 mg% obtained from tail blood or cardiac puncture and measured by the technique of Lauber and Mattice.(5) Fasting blood sugars were not employed in this study, because it was thought that repeated overnight fasts might introduce an irregular influence on the nutritional or

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2. Miller, H. C., *Endocrinology*, 1947, v40, 251.

3. Hultquist, G., *Acta Path. et Microbiol. Scand.*, 1948, v25, 131.

4. Sinden, J. A., and Longwell, B. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 607.

5. Lauber, F. V., and Mattice, M. R., *J. Lab. and Clin. Med.*, 1944, v29, 113.

diabetic state of the animals. In those animals considered permanently diabetic, with two exceptions (c.f. Rats no. 18 and no. 19 below) the criteria for diabetes remained satisfied until the animals were either sacrificed, died, or were removed from the experiment after periods varying from 32 to 425 days.

Results. Of the 25 rats in which permanent diabetes was produced, 12 pregnancies were achieved in 7 rats, 2 of which had 3 pregnancies, 1 had 2 pregnancies and the remainder 1 each. Of the 12 non-diabetic control rats of the same age and strain as the experimental animals, 11 pregnancies were produced in 8 rats over the same period of time. Despite the absence of any effort at treatment of the diabetes, clinical acidosis was not observed in any of the 25 permanently diabetic rats. In no case was insulin administered. Nevertheless, most of the animals remained in apparent good health, with maximum 24 hour water intakes of 200-400 cc and maximum urine outputs of 70-140 cc. Occasional isolated instances of acetonuria were observed. When olive oil was added to the diet, however, acetonuria was frequent and persistent. No blood carbon dioxide studies were performed, but clinical evidence of acidosis failed to appear. Blood sugars in the control group taken under the same non-fasting conditions as in the diabetic group averaged 95 mg% with variation from 79 mg% to 119 mg%. Diabetic animals revealed blood sugars varying from 224 mg% to 650 mg%, with a single exception of one value of 133 mg%. The average of all diabetic blood sugars was 450 mg%.

As previously reported,(1,4) there was disappearance of oestrus vaginal smears for long periods of time and irregular oestrus cycles appeared in females with well established diabetes. In rats made diabetic by subtotal pancreatectomy Richter *et al.*(6) reported the return of normal oestrus activity, when these animals ingested a high fat diet. The main constituent of this high fat diet was olive oil. Richter's observations are in large measure confirmed in our studies. Animals partook of olive oil when it was offered and there was a definite trend toward normal oestrus cycles as a result thereof. In most cases, however, irregularities persisted and in no case did pregnancy occur after the use of olive oil.

Observation of these 12 diabetic and 10 normal pregnancies revealed the following data: (1) The estimated period of gestation varied between 20 and 29 days in the diabetic group and between 20 and 28 days in the control group, with the majority of both groups varying between 20 and 23 days. (2) No significant alteration in the severity of the diabetes was noted during pregnancy or in the puerperium in any of the animals, with the 2 exceptions, *c.f.* Rats no. 18 and no. 19 below. (3) Though urines were not tested routinely for albumin and no blood pressure studies were performed, there was no clinical evidence of toxemia and the pregnancies in the diabetic animals appeared quite as normal as those of the controls. (4) Figures for the number of young per litter as determined by observation were found to be unreliable both in the diabetic and control series, because of the tendency in both groups to some degree of cannibalism. In spite of this, however, no significant difference was noted in the number of offspring per litter in the two groups. (5) No external congenital anomalies were observed at birth in any litters in either the experimental or control series and, so far as clinical observation could determine, the fetuses were grossly of equal size in the two groups. Four offspring of 2 diabetic animals were observed to maturity and these animals proved to be completely normal in all respects. Repeated blood sugar levels were determined in these offspring of diabetic mothers after weaning at 28 to 35 days and no deviation of the blood sugar from normal was observed. (6) Because of the size of the newborn rat it was impossible to measure the blood sugar level and since a clinical diagnosis of hypoglycemia under these conditions was considered valueless, no data were obtained on this phase of the subject. (7) Compared with their prepregnant course and the diabetes of other nonpregnant rats, no alteration in the course of the disease was noted in 5 rats subsequent to

6. Richter, C. P., Schmidt, E. C. H., Jr., and Malone, P. D., *Bull. Johns Hopkins Hosp.*, 1945, v76, 192.

TABLE I.
Data on Pregnant Diabetic Rats.

Rat No.	Duration of diabetes (days)	Alloxan		Glycosuria (non-pregnant period) avg*	Blood sugars (non-pregnant period) range, mg%	Duration of diabetes prior to pregnancy days	Glycosuria (pregnancy) avg*	Blood sugars (pregnancy) (mg%)	Avg
		Doses	mg/kg						
8	351	6	140	4.15 (0.19-12.6) †	330-598	(1) 27 (2) 100 (3) 145	(1) 2.99 (.12-6.7) † (2) 3.40 (.57-6.3) (3) 4.20 (.44-11.8)	(1) 290-432 (2) 400-580 (3) 432 (1)	392 480
16	425	8 1	140 165	0.95 (.00-12.6)	270-522	(1) 50 (2) 124 (3) 207	(1) 0.75 (.0-4.0) (2) 2.87 (.0-8.1) (3) 2.64 (.0-8.0)	(1) 224-400 (2) 380-488 (3) No blood sugar	368 434
17	405	5 2 1	140 180 200	2.85 (.00-5.3)	236-650	(1) 23 (2) 134	(1) 0.58 (.0-3.1) (2) 4.53 (.0-12.1)	(1) 340-400 (2) 402-480	375 471
18	68	3	140	1.28 (.00-2.5)	300-444	53	1.55 (.28‡-3.7)	368 and 26 (2)	
19	32	5 1 1	140 180 205	1.1 (.48-1.7)	250 (1)	4	1.02 (.0-4.0)	444 and 810 (2)	
23	130	1	170	2.56 (.82-5.2)	345-444	4	1.75 (.0-6.1)	328 and 133‖ (2)	
27	274	2	175	4.23 (1.2-11.6)	246-456	16	1.52 (.27-3)	380 single	

* g/24 hr. † Range. ‡ .0 on day of death. ‖ Day before delivering.

single or repeated pregnancies. (8) In Rats No. 18 and 19 of this series a phenomenon was observed which we are at a complete loss to explain. Both of these animals died in hypoglycemia. Neither of them was given insulin at any time. Rat No. 18 was first noted to be somewhat apathetic on the 15th day of her only pregnancy, 68 days after the onset of her diabetes, which prior thereto had not differed from that observed in our other animals. Blood sugar 13 days before death was 368 mg%. Glycosuria in excess of 0.5 g in 24 hours had persisted until the day before death. Shortly after the apathy appeared cyanosis developed. There was bleeding from the nose and vagina. The coat became ruffled and the temperature fell to 99° (R). Five hours after the onset of this train of symptoms the animal was moribund. A cardiac puncture revealed a blood sugar of 26 mg%. Death ensued within 15 minutes. Autopsy was performed immediately. There were no gross abnormalities. Microscopically, the kidneys showed colloid droplet degeneration in both the proximal and distal convoluted tubules. The sections of the eyes showed the presence of incipient cataracts. Well-formed islets of Langerhans were present in sections of the pancreas. There was no necrosis or excessive fat deposition in the adrenals or liver. Sections of the other organs including the pituitary were not remarkable.

Rat No. 19 died with identical symptoms 6 days after delivery of a litter of 9 living young, 32 days after the onset of her disease. Her diabetes had likewise run the usual course. Blood sugar 16 days prior to death was 810 mg%. Following delivery the animal ate no chow and drank little water but devoured her entire litter. She became moribund 8½ hours after the onset of symptoms. Cardiac blood sugar at this time was 22 mg%. Death occurred about 15 minutes later and autopsy was performed immediately. No gross abnormalities could be made out. Microscopically, the only positive finding was the presence of incipient cataracts in sections of the eyes.

Discussion. The results of this study of 12 diabetic and 10 control pregnancies confirm previous reports(1,4) that alloxan diabetes, like pancreatectomy diabetes(6) interferes with normal oestrus. Administration of olive oil to these animals tends to restore a more normal oestrus pattern as demonstrated by Richter(6) for subtotally pancreatectomized animals, but does not influence fertility.

Our results are contrary to those of Davis *et al.*(1) who reported the resorption of fetuses in 4 uncontrolled alloxan diabetic rats and stated that control of the hyperglycemia by insulin, as demonstrated in 7 treated rats, was essential to successful completion of pregnancy with live litters.

Miller(2) reported 10 normal pregnancies in 8 diabetic rats where the average glycosuria per day varied from 0.27 g to 0.5 g, in 3 untreated animals, and from 0.3 g to 1.6 g per day in 7 rats treated with protamine zinc insulin in order to prevent severe glycosuria. No blood sugars were reported. These untreated animals demonstrated mild diabetes as judged by our requirement of a glycosuria in excess of 0.5 g in 24 hours.

Davis *et al.*(1) reported that in untreated alloxan diabetic rats "pregnancy progressed normally until about the 12th day following which the fetus died and was slowly absorbed". Our data, however, do not support this finding and confirm those of Sinden and Longwell.(4) Eleven of the 12 pregnancies studied progressed normally to term with the delivery of normal litters. The need for insulin as stressed by Davis *et al.*(1) did not exist in this series. In fact, the only rat that died during pregnancy manifested a terminal hypoglycemia.

Summary and conclusions. (1) Alloxan diabetes, like pancreatectomy diabetes, interferes with the normal oestrous cycle of rats, and impairs fertility.

(2) Administration of olive oil aids in reversion of the oestrous cycle toward normal, but does not improve fertility.

(3) The course of 11 of the 12 pregnancies reported in this study was uninfluenced by the presence of pre-existing permanent alloxan diabetes.

(4) The course of the diabetes in 5 of the 7 rats observed was not influenced by single or repeated pregnancies.

(5) Two rats died in hypoglycemia, one on the 15th day of pregnancy and one 6 days

post partum. The mechanism of this hypoglycemia is unexplained.

(6) Administration of insulin was not required in order to obtain live, full term litters in 10 of the 11 pregnancies observed.

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Purification of Certain Viruses by Use of Protamine Sulfate. (17535)

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During attempts to improve the immunogenic potency and to increase the purity of Japanese encephalitis vaccine, it was noted that the addition of protamine sulfate to suspensions of infected chick embryo tissue produced a heavy precipitate, and, of particular interest, left almost all of the virus in the clear supernatant fluid. Subsequently, a number of viruses were studied with respect to their behavior in the presence of protamine. It was found that they fell into 2 groups, *i.e.*, those which were precipitated along with the tissue materials as has been observed by others(1,2) and those, like Japanese encephalitis virus, which remained in the supernatant fluid. For this latter group, protamine precipitation provides a rapid, simple and effective means for partial purification.

Methods. A considerable number of orienting experiments indicated that protamine precipitation could be carried out in the following manner: A chilled infected 10% suspension of mouse brain clarified by low-speed centrifugation or a 20% suspension of uncentrifuged chick embryo tissue, pH 7.0 to 7.2, is added to protamine sulfate (salmine) in the proportion of 1.0 ml of tissue suspension for each 5.0 mg of protamine. Either the readily soluble dry protamine powder or a solution of the material is used. A fine precipitate forms immediately on the addition of the tissue suspension to the protamine. The mixture is stored with occasional agitation in the refrigerator for 30 minutes after which the precipitate is separated by low-speed centrifugation at 3000 RPM for 15 minutes in an angle centrifuge. The resultant clear, pink supernatant fluid is decanted and saved; in the present report it is designated as "protamine supernate." Protamine supernates obtained by the method just described develop a granular precipitate during the first 24 hours of storage in the refrigerator. Furthermore, if formaldehyde is added in order to inactivate the virus, a heavy flocculation slowly develops. This flocculation apparently is due to the residual protamine in the preparations. It can be avoided if the excess protamine is removed by precipitating it with heparin. The optimum amount of heparin for this purpose is 3.8 mg per ml of supernate. Less than this amount fails to remove sufficient protamine whereas more produces a finely dispersed precipitate which sediments with difficulty. Thirteen viral agents were subjected to treatment with protamine. Their distribution in the various fractions thus obtained was determined by assaying the infectivity of the materials. In all but 2 instances this was done by intracerebral titration in mice in the usual manner. The exceptions were mumps virus, which was assayed in embryonated eggs by testing for the presence of viral hemagglutinins in the chorioallantoic fluids, and one strain of vaccinia virus, which was titrated on the chorioallantoic membrane.

Results. Action of protamine on infected suspensions. The viral agents which remain in the clarified supernatant fluid after treatment with protamine are listed in Table I, as are those which are precipitated along with tissue materials by this treatment. Others

1. Chambers, L. A., and Henle, W., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 481.

2. Bawden, F. C., and Pirie, N. W., *Proc. Roy. Soc., Series B,* 1937, v123, 274.