The Effect of Protamine Zinc Insulin on the Outcome of Pregnancy in the Normal Rat

E. J. Love, M.D., R. A. H. Kinch, M.B., and J. A. F. Stevenson, M.D.
London, Ontario, Canada

SUMMARY

Intermittent hypoglycemia was produced in normal pregnant rats by the administration of 0.5 I.U. of Protamine Zinc Insulin at twelve-hour intervals during either the last two weeks or the final week of pregnancy. The animals treated during the final week of pregnancy delivered significantly lighter litters, with a reduction in the weight of the living pups, and with a significant increase in the incidence of stillbirths which were significantly lighter than the pups delivered to the untreated controls. The exact cause of the growth retardation and the excessive loss are as yet unknown. Two alternate hypotheses are suggested: (1) that growth retardation occurred as the result of relative inanition of the fetus associated with the maternal hypoglycemia, less carbohydrate being available for fetal nutrition and growth, or (2) that with intermittent hypoglycemia there was less stimulation of the fetal pancreatic islets and of the production of insulin with its growth-promoting properties.

The animals treated with insulin for the last two weeks of pregnancy delivered litters of equal number and weight as the controls; the living pups were of equal weight compared to the controls; the number and weight of the stillborn pups was significantly increased. To explain this unexpected finding, it is suggested that in time adrenal cortical hyperfunction occurs secondary to intermittent bouts of hypoglycemia and that the normal or excessive growth is the result of excessive amounts of maternal adrenal corticoids.

The causes of excessively large fetuses, late intrauterine death, and increased neonatal mortality which often accompany pregnancy in the diabetic woman are still unknown. In a previous study,[16] we found that the alloxan-diabetic rat delivered a smaller and lighter litter but significantly heavier individual pups than the control animal. Treatment of these alloxan-diabetic rats during pregnancy with Protamine Zinc Insulin in amounts sufficient to maintain a glucose-free urine resulted in the delivery of pups which were significantly lighter than those either of the untreated diabetic or of the normal rats and in a marked increase in the stillbirth rate. The purpose of the present study was to determine the effect of treatment with Protamine Zinc Insulin on the pregnancy and offspring of normal animals.

From the Departments of Physiology and Obstetrics and Gynecology, Faculty of Medicine, University of Western Ontario, London, Ontario, Canada.

METHODS

Virgin female Sprague-Dawley rats weighing 200 to 250 gm., given food and water ad libitum, were placed with males of proven fertility and were isolated when sperm were found in the vaginal smear. Some of the animals were given Protamine Zinc Insulin (0.5 I.U. s.c. every twelve hours) during the last two weeks of pregnancy; others were given insulin (0.5 I.U. s.c. every twelve hours) for the final week of pregnancy. On random days during pregnancy the blood sugar was measured on samples of tail blood by the microversion of the method of Nelson[19] and Somogyi[22] as modified by King[13] and Natelson,[18] at one, two, three, six and twelve hours after the administration of insulin.

Some of the animals were allowed to deliver spontaneously. The pups were weighed when found and fasted for two hours. Blood for glucose determination was then obtained from the pups by decapitation. The carcasses of all the live and of all the stillborn pups in each litter were separated into two groups, ground to homogeneity and the composition determined: the water by a modification of the method of Fischer et al.,[5] the protein by the method of Ma and Zuazaga,[17] and the ash by combustion.

The remaining insulin-treated animals were delivered by cesarean section, under Nembutal anesthesia, on the twenty-first day of pregnancy. The fetuses, their placenta and the maternal adrenals were weighed.

Some of the surviving control animals were remated and, when pregnant again, were isolated and given food and water ad libitum. Protamine Zinc Insulin (1 I.U., s.c. every twelve hours) was administered from the eighth day of pregnancy until delivery by cesarean

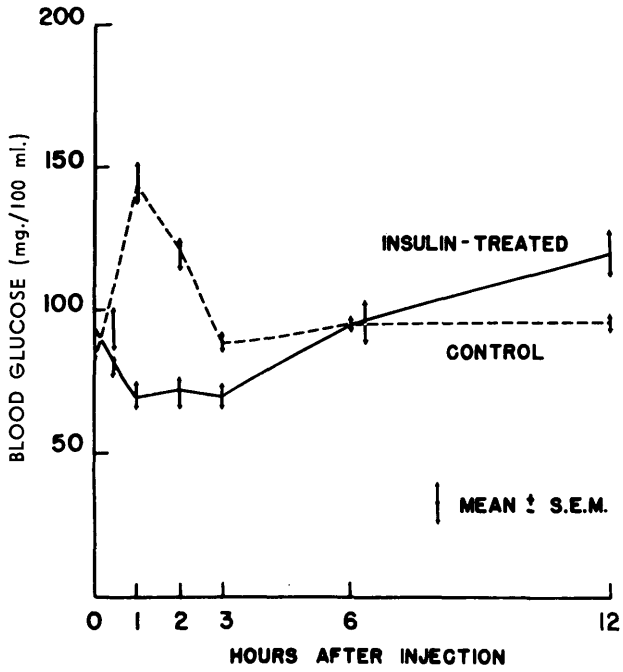

FIG. 1. The effect of Protamine Zinc Insulin (0.5 I.U.) injected subcutaneously at time 0 (10 a.m.) on the blood sugar of normal pregnant rats given food and water ad libitum.
— — — — — Control
———— Insulin-treated

section on the nineteenth day. At cesarean section after four hours without food, a maternal blood sample was obtained for glucose determination, the fetuses were weighed and fetal blood was obtained by decapitation for glucose determination.

RESULTS

Protamine Zinc Insulin (0.5 I.U.) given subcutaneously every twelve hours (10 a.m. and 10 p.m.) during the last two weeks, or the final week of pregnancy, caused a significant reduction in the blood sugar for three hours following injection. Within six hours the blood sugar of the treated animals had returned to the level observed in the control animals and, just before the next injection, it was higher than that of the controls (figure 1).

The control animals delivered 10.3 ± 0.43 (S.E.M.) pups per litter with a mean litter weight of 63.7 ± 7.60 gm. (table 1). The animals treated during the final week of pregnancy delivered fewer pups and significantly lighter litters. The live pups were lighter than the control pups but the difference was not significant. More than one third of the pups born to these insulin-treated animals were stillborn, and these stillborns were significantly lighter than either their live litter mates or the stillbirths born to the control animals.

On the other hand, the only differences between the litters born to the controls and to the animals treated for the last two weeks of pregnancy were a significant increase in the number of stillbirths per litter and a significant increase in the weight of these stillbirths. There was no difference in the length of gestation, as calculated from the date of observing sperm in the vaginal smear to the date of delivery, between the control and insulin-treated animals.

When fasted, the pups of the insulin-treated animals had significantly lower blood sugars two hours after birth than the pups of the control animals. The lowest blood sugars were observed in the pups delivered to the animals treated with insulin for the final weeks of pregnancy (table 1). There was no significant difference in the composition of the carcasses of the pups born to any of the groups.

The animals treated with insulin and delivered by

TABLE 1

Litters born spontaneously to normal and insulin-treated rats

	No. pups in litter	Litter weight (gm.)	Mean pup weight/litter (gm.)	Live pup weight (gm.)	Two-hr. blood sugar (mg. per 100 ml.)	Stillbirth Per cent litter	Stillbirth Weight (gm.)
Control	10.3±0.43* (20)†	63.7±7.60	6.18±.113	6.22±.041 (184)	133±4.3 (113)	9.1	5.85±.159 (22)
PZI—0.5 I.U.	9.0±0.66	52.2±9.38	5.90±.235	6.08±.060	81±5.2	33.6	5.25±.228
q 12 h. × 1 wk.	(10)			(59)	(26)		(31)
PZI—0.5 I.U.	10.0±1.03	62.5±6.35	6.32±.191	6.20±.066	96±6.7	30.6	6.38±0.169
q 12 h. × 2 wk.	(10)			(72)	(25)		(28)

*Mean ± S.E.M.
†Number of observations
Underlined values significantly different from Control ——— P<0.05; ——— P<0.01; ═══ P<0.001.

TABLE 2

Maternal weight gain, maternal adrenal weight, fetal and placental weight at cesarean section on the twenty-first day of pregnancy in normal and insulin-treated rats

	Maternal weight gain (gm.)	Maternal adrenal weight (mg.)	Fetal weight (gm.)	Placental weight (gm.)	Ratio placental/fetal weight
Control	107±5.2* (10)†	56.2±4.08 (10)	5.37±.078 (112)	0.58±.006	0.110±.0019
PZI—0.5 I.U. q 9.12 h. × 2 wk.	85±8.6 (6)	60.0±5.77 (6)	5.41±.090 (62)	0.56±.014	0.105±.0032

*Mean ± S.E.M.
†Number of observations.

TABLE 3

Maternal and fetal blood sugar and the fetal weight at cesarean section on the nineteenth day of pregnancy in normal and insulin-treated rats

	No. litters	Fetal weight	Blood sugar (mg. per 100 ml.) maternal	Blood sugar (mg. per 100 ml.) Fetal	Blood sugar (mg. per 100 ml.) Ratio maternal/fetal
Control	10	2.48±0.028* (95)†	95±8.4 (9)	56±4.2 (44)	2.05±0.140
PZI—0.5 I.U. q 12 h. D8—D19	5	2.49±0.032 (60)	66±8.3 (5)	67±4.8 (37)	1.19±0.087

*Mean ± S.E.M.
†Number of observations.
Underlined values significantly different from control ═══ $P<0.001$.

cesarean section on the twenty-first day of pregnancy gained slightly less weight than the untreated control animals (table 2). Their adrenals were slightly heavier than the controls, but the difference was not significant. There was no real difference in the mean fetal or placental weights, or in the mean placental-fetal weight ratio of the insulin-treated and the control animals (table 2).

Five rats were given Protamine Zinc Insulin (1 I.U. every twelve hours) for twelve days beginning on the eighth day of their second pregnancy. Although the food and water intakes of these animals were slightly increased, the difference was not significant. At cesarean section, the mean blood sugar of the animals given insulin four hours previously was significantly less than that of the control animals, but there was no difference in the fetal blood sugar of the two groups (table 3). Nor was there any difference in the mean weight of the fetuses delivered to the control and insulin-treated animals (table 3).

DISCUSSION

We have reported that uncontrolled alloxan-diabetic rats delivered fewer pups and lighter litters with an increased incidence of stillbirths.[16] The mean weight of the individual pups, especially the stillborn, was significantly heavier than that of the pups born to the control animals. However, when treated with Protamine Zinc Insulin in amounts sufficient to maintain a glucose-free urine, the diabetic animals delivered an even greater proportion of stillbirths and their pups, including the stillborn, were significantly lighter. Davis et al.[3] and Ferret et al.[4] found that the offspring of insulin-treated alloxan-diabetic rats were of normal weight, but neither of these groups attempted to control the diabetes strictly. In our study, control was severe enough to result in the death of several animals before delivery. This observation and that of Cohen[2] who found that the degree of urinary glucose parallels the blood sugar of alloxan-diabetic rats, suggest that our insulin-treated diabetics were in fact subjected to intermittent bouts of hypoglycemia.

In the present investigation normal pregnant animals were given Protamine Zinc Insulin and as a result were subjected to intermittent bouts of relative hypoglycemia. When insulin was administered for the final week of pregnancy, there was a slight reduction in the weight of the live-born pups, a marked increase in the number of stillborn pups, and these stillborn pups were significantly lighter than either their live litter

mates or the stillborn of the untreated animals. These results are consistent with those of Lichtenstein.[15]

There are several possible explanations for retardation of fetal growth and the excessive fetal loss in the litters of insulin-treated diabetics and of normal animals treated with insulin for the final week of pregnancy. Since it has been shown that insulin does cross the placental barrier,[14] these effects might be due to a direct toxic effect of exogenous insulin on the fetus. However, it seems more likely that these effects are the direct or indirect result of maternally-induced hypoglycemia in the fetus.

Britton[1] and Friedgood et al.[6] have shown that the fetal blood glucose parallels the maternal blood glucose, but at a slightly lower level, and this is apparent in the positive correlation between the maternal and the fetal blood glucose levels in diabetic rats.[7,11,12] Intermittent maternal hypoglycemia, whether occurring in the diabetic or normal rat, would cause hypoglycemia in the fetus. These bouts of hypoglycemia may well have been the cause of the increased death rates in these litters. The decreased fetal growth may also be a direct result of intermittent hypoglycemia resulting in less carbohydrate being made available for fetal nutrition and growth. The findings of lowered blood glucose levels in the pups of the insulin-treated animals two hours after birth would support this hypothesis.

An alternate but compatible hypothesis is that relative fetal hypoglycemia, paralleling maternal hypoglycemia, results in a relative lack of stimulation of the fetal pancreatic islets. Histological studies have indicated that the pancreatic islets begin to develop on the thirteenth day, the secretory granules of the beta cells become distinguishable on the nineteenth day, and secretory activity is apparent on the twenty-first day of pregnancy.[9] The islets of pups delivered of rats treated with large doses of Protamine Zinc Insulin are, however, almost devoid of beta cells.[20] In the absence of adequate stimulation of the fetal pancreas inadequate amounts of fetal insulin would be produced. Since insulin has been shown to have growth-promoting properties[21] the decreased growth may, therefore, be the result of the relative lack of insulin in these hypoglycemic pups.

Neither of these hypotheses will, however, explain why fetal growth retardation did not occur in the animals treated with insulin for two weeks. These animals and their fetuses were also subjected to intermittent bouts of hypoglycemia. Yet, when delivered by cesarean section on Day 21, their fetuses were equal in weight to those of the controls, and when delivered spontaneously the living pups were of normal weight and the stillborn of excessive weight. It may be that the animals treated for two weeks were able to adapt to some extent to these bouts of intermittent hypoglycemia, supplying somewhat higher blood glucose levels and therefore more carbohydrate to the fetus during the last few days of pregnancy when there is particularly rapid growth. That this may be the case is suggested by the somewhat higher neonatal blood glucose levels for the pups of the animals treated with insulin for the last two weeks of pregnancy compared to those treated for only the final week of pregnancy. Perhaps, as suggested by Tepperman et al.[24] and others[8,23,25] these bouts of insulin-induced hypoglycemia were sufficient stimulus in time to cause adrenocortical hyperfunction. Hoet[10] has suggested that the excessive fetal growth in diabetic pregnancies is the result of the excessive maternal corticoids. If adrenocortical hyperfunction did occur and if corticoids in excess will cause excessive growth, we could explain the relative increase in fetal growth observed in the animals subjected to repeated bouts of insulin-induced hypoglycemia. Presumedly, high levels of maternal corticoids would also suppress the development of the fetal adrenal cortex. This would then explain the lethargy and lowered fasting blood glucose subsequently observed in the living pups of the insulin-treated animals. It would also explain the excessive fetal loss, the larger pups dying during or immediately following delivery as the result of adrenal insufficiency.

SUMMARIO IN INTERLINGUA

Le Resultato de Insulina a Protamina-Zinc Super le Resultato del Pregnantia de Rattas Normal

Hypoglycemia intermittente esseva producite in normal rattas pregnante per le administration de 0,5 U.I. de insulina a protamina-zinc a intervallos de dece-duo horas durante (a) le ultime duo septimanas o (b) le ultime un septimana del pregnantia. Le animales tractate durante le ultime septimana del pregnantia parturiva significativemente plus leve portatas, con reduction del pesos del superviventes e con un significative augmento del incidentia de mortenascentias in le quales le pesos fetal esseva significativemente inferior a illos in le casos de non-tractate rattas de controlo. Le precise causa del retardo in crescentia e del excesso in perditas es ancora incognoscite. Es formulate duo separate hypotheses, i.e. (1) que le retardamento del crescentia occurreva como consequentia del relative inanition del feto in association con le hypoglycemia materne in tanto que reducite quantitates de hydrato de carbon

esseva disponibile pro nutrition e crescentia o (2) que in le presentia de hypoglycemia intermittente il habeva minus stimulation del insulas pancreatic del fetos e assi del production de insulina le qual possede proprietates de promotion crescential.

Le animales que esseva tractate con insulina durante le ultime duo septimanas del pregnantias parturiva portatas equal in numeros e pesos con illos del non-tractate animales de controlo. Le supervivente juvenes habeva le mesme peso como le juvenes ab matres de controlo, durante que le numero e le peso del mortenatos esseva significativemente augmentate. Pro explicar iste inexpectate constatation, le these es presentate que hyperfunction adreno-cortical occurre chronologicamente secundari a intermittente episodios hypoglycemic e que le crescentia normal o excessive es le resultato de normal o excessive quantitates de materne adreno-corticoides.

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