THE INSULIN ACTIVITY OF THE BLOOD PLASMA IN FEMALE AND NEWBORN RABBITS DURING ALLOXAN DIABETES INDUCED AT DIFFERENT PERIODS OF PREGNANCY

L. L. Liberman and S. E. Drizgalovich-Egorova

From the Laboratory of Endocrinology (Scientific Director – AMN SSSR Active Member Prof. V. G. Baranov) and the Laboratory of Pathophysiology (Head – Prof. N. L. Garmasheva) of the Institute of Obstetrics and Gynecology (Dir. – Prof. M. A. Petrov-Maslakov), AMN SSSR, Leningrad

(Presented by AMN SSSR Active Member V. G. Baranov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 53, No. 2, pp. 63-66, February, 1962
Original article submitted March 14, 1961

The course of diabetes mellitus usually deteriorates during pregnancy, but in some cases there is an improvement at the end of pregnancy which is usually associated with increased formation of insulin by the fetus and the passage of this insulin through the placenta to the maternal blood. Decompensated diabetes mellitus during fetuses, often leads to the death of the fetus, though the weight of the latter is often high and the fluid-fat-protein ratio normal [9]; in these cases, hyperplasia of the pancreatic insular apparatus is observed in the fetuses, and newborn young [2, 3].

Indirectly, these facts suggest that the increased fetal production of insulin under conditions of maternal diabetes mellitus may have something to do with the increased weight and death of some of the fetuses and newborn young. Direct determination of the insulin activity in the blood of newborn young could help answer this question. We could not find, however, any data on insulin activity in the blood of newborn mammals in the literature. In one work conducted with people [8], no insulin activity could be detected in the umbilical blood of newborn bodies, and the activity of their mothers' blood was normal. It should be noted, however, that insulin can pass through the placenta in both directions [7, 10].

The purpose of this work was to study the insulin activity in the blood of female and newborn rabbits under conditions of maternal alloxan diabetes. The experiments were performed on female rabbits because in these animals, the administration of alloxan during pregnancy causes only a brief diabetes, rapidly compensated without the use of insulin. This makes it possible to establish the period in fetal development at which diabetes in the mother can cause hyperfunction of the insular apparatus in the fetus. It is important to realize that although alloxan passes rapidly through the placenta, it does not damage the insular apparatus of the fetus [1, 9]. In this work, alloxan was administered the 12th day of pregnancy, i.e. during placentation, or the 22nd day, i.e. during the period in which the islets form in the fetal pancreas.

EXPERIMENTAL METHODS

A number of experiments were performed with each of the pregnant rabbits (2810-3910 g) used in the work; the animals were divided into three groups: 1) control animals (11); 2) rabbits given alloxan the 12th day of pregnancy (11); 3) rabbits given alloxan the 22nd day of pregnancy (10). Diabetes was induced (S. E. Drizgalovich-Egorova) by the intravenous injection of alloxan in a dose of 200 mg/kg. The blood sugar and the presence of sugar and acetone in the rabbits' urine were determined several times. The living baby rabbits and the dead (including those which died immediately after birth) were weighed immediately after delivery. The insulin activity and sugar were determined (L. L. Liberman) in the venous blood of the mother rabbits and in a mixture of the blood taken from all the newborn rabbits after their decapitation. The blood was taken as soon after delivery as possible.

Blood sugar was determined by the arseno-molybdate method [6]. The insulin activity of the defibrinated plasma, diluted five times, was determined according to the glucose absorption of rat's isolated epididymal fat [5]. After the rapid removal, without cooling, of this fat from a decapitated rat, one batch was incubated in a carbonate buffer [4] with 200 mg% gelatin, a second batch, in the diluted plasma. The glucose concentration in the media was 400 mg%.

The gaseous environment was made up of 95% air and 5% carbon dioxide. Incubation was done in a Warburg's apparatus (shaken 120 times per minute) for three hours at 37°. The glucose concentration in the medium was then determined and the glucose absorbed by the fatty tissue computed and expressed in milligrams of glucose per 1 g fat for three hours.

TABLE 1. Number and Average Weight of Newborn Rabbits

Group	Number of baby rabbits		Average no. of progeny per female		Average weight of baby rabbits (in g)		Significance of weight difference between living and dead baby rabbits
	living	dead	living	dead	living	dead	
First (control	61	—	5,6	—	51,6±3,0	—	—
Second	38	24	3,4	2,2	43,2±9,7	55,5±15,1	D<0,05
Third	32	25	3,2	2,5	56,5±11,2	64,3±12,9	D<0,05
					D (1—2)<0,001	D(2—3)<0,05	
					D (1—3)<0,05		
					D (2—3)<0,001		

Note: The figures in parentheses give the numbers of the groups being compared.

The insulin effect was determined from the difference in the amounts of glucose absorbed by the fatty tissue from the diluted plasma and the buffer. Each determination was made with five rats, the arithmetic mean of these determinations being taken as the result.

TABLE 2. Glycemia and Insulin Activity of Blood Plasma in Female and Newborn Rabbits

Group	Insulin activity of blood (in micro-units per ml)			Blood sugar (in mg%)		
	females	progeny	significance of difference	females	progeny	significance of difference
First	809 ± 159	707 ± 74	D > 0,05	120 ± 3	73 ± 5	D < 0,001
Second	1,057 ± 150	766 ± 213	D < 0,01	133 ± 10	86 ± 6	D < 0,001
Third	1,225 ± 121	1,214 ± 226	D > 0,5	151 ± 13	77 ± 14	D < 0,001
	D (1-2) < 0,01	D (1-2) > 0,4		D (1-2) < 0,01	D (1-2)	< 0,001
	D (1-3) < 0,001	D (1-3) < 0,001		D (1-3) < 0,001	D (1-3)	> 0,4
	D (2-3) < 0,05	D (2-3) < 0,01		D (2-3) < 0,01	D (2-3)	> 0,1

These experiments were done many times in the course of the work; the effect of 12 different concentrations of insulin (from 10 to 100,000 international units per milliliter) was determined in order that a curve showing how the insulin effect depended on the concentration of insulin in the medium might be constructed. The insulin activity of the diluted plasma was computed on the basis of its insulin effect as shown by the curve, then corrected for undiluted plasma.

EXPERIMENTAL RESULTS

Diabetes mellitus, hyperglycemia (180-370 mg%) and glycosuria developed in all the female rabbits after the administration of alloxan, and acetonuria was observed in a number of cases. The blood sugar content in most of the animals decreased to the upper normal limit after 3-7 days and was normal at the time of delivery, at which time sugar and acetone were no longer present in the urine. High hyperglycemia at the time of delivery was only observed in five rabbits; three of these animals died the day of delivery, and we were unable to obtain blood for analysis from two of them. The data on these rabbits are given separately and were not used to compute the average group values. Table 1 gives data showing the number and average weight of the newborn rabbits.

Table 2 shows the insulin activity of the blood plasma and the hyperglycemia in the female and newborn rabbits (excluding, as explained above, the cases of the mothers with high hyperglycemia).

First, let us consider the data on the females in which hyperglycemia was still high at the time of delivery. One rabbit in the second group died during delivery. Her progeny had a blood sugar content of 54 mg%, and the insulin activity of their blood was 110 micro-units per ml. A second rabbit died two hours after delivery. The insulin activity of her blood was below the detectible level (i.e. less than 50 micro-units per ml), and the blood sugar content was 280 mg%; these indices were 590 micro-units per ml and 154 mg% respectively for the newborn rabbits. In the third female, the blood sugar level was 239 mg% after delivery, the insulin activity, 50 micro-units per ml; in her progeny, these indices were respectively 108 mg% and 400 micro-units per ml. One rabbit in the third group died during delivery; the blood sugar content of the newborn rabbits was 46 mg%, the insulin activity of their blood, 1040 micro-units per ml. The highest insulin activity of the blood was observed in another female with glycemia equal to 294 mg% (2900 micro-units per ml); for her progeny, these indices were respectively 56 mg% and 1300 micro-units per ml.

Observation of the animals for 5-10 months after delivery showed no elevation of the blood sugar level in any of the surviving females, the two with hyperglycemia after delivery included.

The following conclusions can be drawn from the data obtained.

The insulin activity of the blood of normal newborn rabbits is the same as that of the venous blood of their mothers.

The blood of all the experimental female rabbits in which no hyperglycemia was present after delivery disclosed insulin activity. This activity was high in the rabbits which received alloxan the 12th day of pregnancy and still higher in those which received it the 22nd day, suggesting the restoration and even frequent hyperfunction of the insular apparatus of these rabbits. The fact that further observation disclosed no hyperglycemia in these rabbits indicates that the insulin activity of their blood actually reflected the function of their insular apparatus and was not due to the passage of insulin from the fetus through the placenta.

The administration of alloxan to female rabbits the 12th day of pregnancy does not change the insulin activity in the blood of their newborn young. But there is a considerable increase in the insulin activity in the blood of the progeny when alloxan is administered the 22nd day of pregnancy. This means that the insufficiency of the insular apparatus in the females, at the end of pregnancy although brief and compensated by the time of delivery, does cause hyperfunction of the fetal insular apparatus. This hyperfunction probably has something to do with higher average weight of newborn young in this group as compared with the control. However, diabetes induced in a female by alloxan administered the 12th day of pregnancy is already compensated to a large extent by the time the insular apparatus of the fetus begins to function and therefore is not reflected in the insular function of the fetus at the time of delivery.

In the case of the several female rabbits in which considerable hyperglycemia was observed after delivery, the insulin activity of their progeny's blood was normal, while that of the maternal blood was either very low or entirely lacking. This means that the passage of fetal insulin to the maternal blood was negligible in these animals.

Further observation of the female rabbits after delivery showed the presence of insulin in their blood plasma to be a favorable prognosis of complete recovery from diabetes regardless of the level of glycemia.

SUMMARY

Insulin activity of the blood in female and newborn rabbits was determined at the immediate post-partum period by the method of glucose uptake by the isolated epididymis fat of rats. In control animals the insulin activity of the blood in female rabbits was 809 μ U/ml. Upon administration of alloxan on the 12th day of prenancy in a dose of 200 mg/kg,diabetes was compensated by the time of delivery in almost all the rabbits; the insulin activity of the blood was increased (1057 μU/ml). In rabbits which received a similar dose of alloxan on the 22nd day of pregnancy, the insulin activity of the blood after delivery was 1225 μU/ml and in newborn rabbits 1214 μU/ml. Consequently, alloxan diabetes, existing for a brief period of time and compensated by the time of delivery, may cause hyperfunction of the insular apparatus of the fetus only if it existed at the moment of functioning of the fetal insular apparatus, i.e. at the end of pregnancy.

LITERATURE CITED

1. C. E. Friedgood and A. A. Miller, Proc. Soc. exp. Biol. (N. Y.), Vol. 59, p. 61 (1945).
2. B. E. Frye, J. Morph. Vol. 101, p. 325 (1957).
3. W. P. U. Jackson and N. Woolf, Diabetes, Vol. 7, p. 446 (1958).

4. H. A. Krebs and K. Henseleit, Hoppe-Seyler's Z. physiol. Chem. Bd. 210, S. 33 (1932).
5. D. B. Martin, A. E. Renold, and Y. M. Dagenais, Lancet, Vol. 2, p. 76 (1958).
6. N. Nelson, J. biol. Chem. Vol. 153, p. 375 (1944).
7. J. Olow, Biochem. Z. Bd. 217, S. 475 (1930).
8. R. Santos, R. McCance, and P. Randle, Nature, Vol. 176, p. 115 (1955).
9. F. Solomon, Diabetes, Vol. 8, p. 45 (1959).
10. P. Spoto, Minerva ginec. Vol. 3, p. 72 (1954).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
