EXPERIMENTAL PREDIABETES IN RATS AND ITS EFFECT ON THE OUTCOME OF PREGNANCY AND THE WEIGHT OF THE FETUSES

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UDC 618.3-06 : 616.379-036.3-092.9]-036.8 : 618.291

Considerable attention has recently been paid to prediabetes – the earliest of the known stages of diabetes – in man. At this stage prophylactic measures can be used to prevent the development of a latent or frank form of diabetes mellitus.

In prediabetes the fasting blood sugar is normal, glycosuria is absent, and the sugar curve after a glucose load retains its physiological character. Prediabetes is manifested in women by a pathological course of pregnancy and by birth of a large fetus [5-7]. This is a largely hypothetical disease, because a pathological course of pregnancy and birth of a large fetus are observed not only in diabetes; the conclusion that these disturbances are in fact manifestations of diabetes may be made retrospectively (when latent or frank diabetes is detected). Persons with a family history of diabetes, and also obese subjects, among whom diabetes is particularly common, may be suspected to some extent of having prediabetes.

The object of the present investigation was to obtain in rats a model of latent insufficiency of the insular β-cells, not diagnosable clinically, and corresponding in its diagnostic signs to prediabetes in man, and to study the course of pregnancy and the weight of the fetuses in these animals.

The effect of experimental diabetes on the course of pregnancy and the weight of the fetuses has been investigated many times, but always in animals with frank diabetes. Most investigators observed that the diabetic animals gave birth to large fetuses, and that they died in various stages of pregnancy [5, 7]. However, some investigators [8], ruling out the possibility of pregnancy going beyond full term by terminating it artificially 519 h after its beginning, pointed out that the weight of the fetuses in animals with diabetes was smaller than in controls. At the same time, reports have been published [10] indicating the greater weight of the fetuses in severe diabetes and their smaller weight in mild diabetes.

EXPERIMENTAL METHOD

To obtain a model of prediabetes in rats, the starting point was an investigation undertaken by one of the authors (I. M. S.) showing that sexually immature albino rats possess higher resistance to alloxan than sexually mature rats, and the developing diabetic disturbances in most animals disappeared for good after 4-25 days [1].

Experiments were carried out on 388 sexually immature female Wistar rats whose date of birth was known. Alloxan was injected subcutaneously as a 5% freshly prepared aqueous solution in a dose of 170-500 mg/kg depending on age. The animals were weighed daily or once ever 2-4 days. The blood sugar was determined by the Hagedorn–Jensen method once ever 3-7 days. After the animals were able to feed themselves (at the age of 3 weeks) they were kept in special cages in which the 24-hour urine could be collected. The sugar content of the urine was determined and qualitative tests carried out for acetone and protein.

A glucose tolerance test, in which glucose was give by mouth in a dose of 400 mg/100 g, was carried out on all animals developing temporary alloxan diabetes in the past followed by clinical recovery (with a persistent normoglycemia and aglucosuria). Blood was taken before administration of the glucose and 1 and 2 h thereafter. The results obtained were compared with the blood sugar curves of 30 healthy rats.

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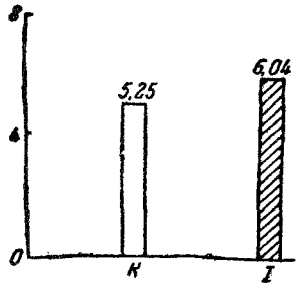

Mean weight (in g) of rat fetuses extracted by Caesarian section from females with alloxan prediabetes (I). K) Control.

Animals with a normal glucose tolerance test were used in the experiments. A blood sugar level higher than physiological after administration of glucose was found in only three of the 44 rats.

In all the animals of both the experimental and the control groups, vaginal smears were examined every hour after mating with males. The moment of discovery of spermatozoa in the smears was taken as the possible beginning of pregnancy. The fetuses were extracted from the pregnant animals 522 h after discovery of spermatozoa in the vaginal smear by caesarian section and their weight was measured.

EXPERIMENTAL RESULTS

In the albino rats of a local breed, developing a temporary form of alloxan diabetes when sexually immature, and subsequently showing clinical recovery, pregnancy often terminated in the birth of large and nonviable fetuses. The mean weight of 232 newborn rats born from these animals was 7.3 g (6-12 g), while the mean weight of 274 newborn rats born from healthy animals was 6.5 g (5-8 g); the difference is statistically significant ($P < 0.05$). The newborn animals of the experimental group, the weight of which exceeded the upper limit of weight (8 g) of the newborn rats of the control group, accounted for 38.3%, while the premature and nonviable fetuses in the experimental group accounted for 20.9% of the total.

Of the 119 rats born from mothers with a frank chronic form of alloxan diabetes, 69 were full-term and 50 were premature and nonviable. The mean weight of the newborn full-term rats of this group was 7.82 g (6.4-12.3 g), while the mean weight of the premature rats was 4.1 g (3.0-5.7 g). The weight of 40% of the full-term rats of this group was higher than the limiting weight of the rats born from healthy mothers. However, in this series of investigations on the rats no glucose tolerance test was carried out, and no data are available indicating the form of disturbances present — whether latent diabetes or prediabetes. In addition, the duration of pregnancy was not taken into account and the possibility of postmaturity could not be excluded.

A comparison was made of the weight of 83 fetuses from mother rats which had temporary alloxan diabetes while sexually immature, and the weight of 81 fetuses of healthy mother rats. All the females were of the same age (4.5-6 months), and the pregnancy studied in these animals was the first. The figure shows that the mean weight of the fetuses in the experimental group was 6.04 ± 0.06 g (4.88-7.3 g), compared with 5.25 ± 0.06 g (4.18-6.00 g) in the control group; the differnce is statistically significant ($P < 0.001$). In 40 of the 83 fetuses of the experimental group of females the weight was above the upper limit of weight of the fetuses (6 g) of the control group of females. From all the fetuses of the rats of the experimental group, those which had died and were in various stages of maceration were extracted. No dead fetuses were found in the control females.

DISCUSSION OF RESULTS

A stable normal blood sugar level and a persistent absence of glucosuria during a normal glucose tolerance test, the development of large fetuses and their frequent intrauterine death — all these suggest that this form of alloxan pathology was prediabetes.

The results of one of the authors' previous investigations,* in which some animals with the same form of latent pathology developed frank diabetes as a result of parturition, of the addition of sugar to the diet, and also of disturbances of higher nervous activity caused by application of strong external stimuli, confirm that in these rats a latent pathological condition of the β-cells of the pancreatic insular system was in fact present.

The pathology of fetal development in rats with a latent form of alloxan disturbance and the analogous pathology in rats with frank chronic alloxan diabetes undoubtedly share the same pathogenesis. The only difference lies in the severity of the lesion in the islet-cells producing insulin.

One of the factors leading to the development of large fetuses in frank diabetes must be the high blood sugar concentration. This is confirmed by data [4] showing that the prolonged administration of glucose to pregnant rats leads to the development of larger fetuses. On the other hand, administration of insulin to pregnant animals lowers

* These results were reported at a Congress of Internists in Yugoslavia in 1964.

the weight of the fetuses [9]. In diabetes, the glucose passing through the placenta probably cause compensatory hyperplasia and hypertrophy of the islet apparatus [3, 11]. The higher secretion of insulin by the islet-cells of the fetus in the presence of maternal diabetes has been clearly demonstrated experimentally [2].

However, probably not only glucose, entering the fetus in large quantities from the mother, is concerned in the genesis of the hyperplasia and hypertrophy of the islets, but also other products of the disturbed metabolism. This suggestion is based on clinical observations on patients with prediabetes and the results of earlier experiments undertaken by the authors on the study of experimental prediabetes in which, while a normal stable blood sugar level and a normal glucose tolerance were preserved, large fetuses were born and the course of pregnancy was pathological.

The alloxan model of this latent pathological processes used in these experiments suggests that the development of large fetuses in these animals was due to a changed, although not clinically detectable, level of production of insulin.

SUMMARY

Experiments were carried out on Wistar rats which sustained at a sexually immature age a transient form of alloxan diabetes with subsequent stable elimination of hyperglycemia and glucosuria and with a normal test for tolerance to glucose. The average weight of 232 newly-born rats from these test rats was 7.3 (6.0-12.0), the average weight of 274 newly-born rats from healthy mothers being 6.5 (5.0-8.0). The difference is significant ($P = 0.05$).

In another series of experiments, the term of pregnancy was taken into consideration to rule out the possible factor of prolonged pregnancy in diabetes-affected animals (Wistar rats). In pregnant animals 522 hours after their insemination fetuses were extracted by caesarean section and weighed. The weight of fetuses under these experimental conditions was 6.04 whereas in the control group it was 5.25; the difference is significant ($p < 0.001$).

The model of latent pathology used in our experiments gives one reason to consider that the cause of the development of large fetuses in these animals is a changed, although clinically unidentifiable, production of insulin.

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