Stimulatory and Inhibitory Effects of Glucose and Insulin on Rat Blastocyst Development In Vitro

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The effect of glucose and insulin on the in vitro development of the rat preimplantation embryo was studied by incubating rat blastocysts recovered on days 5 or 6 of pregnancy in the absence or presence of increasing levels of glucose and/or insulin for 24 or 48 h. A differential cell-staining method allowed the separate counting of inner cell mass (ICM) and trophectoderm (TE) cells at the end of the incubation period. In a high-glucose medium (17 mM), ICM and, to a lesser extent, TE developments were significantly and irreversibly inhibited. Low insulin concentrations (3 pM) stimulated ICM and TE development in the presence of 1.1 or 6 mM glucose. Higher insulin levels (30–600 pM) in a 6-mM glucose medium, resulted in a dose-dependent inhibition of ICM and, to a lesser extent, TE development after both 24 and 48 h. This insulin-induced inhibition was reversible if insulin was removed from the medium after 24 h. In the absence of glucose in the medium, insulin was neither stimulatory nor inhibitory on ICM growth. Dead-cell occurrence in ICM after a 48-h incubation increased with increasing glucose concentration in the medium. Insulin alone did not increase dead-cell number but enhanced the effect of glucose. These results show that, in the presence of glucose, insulin might be stimulatory (at low concentrations) or inhibitory (at higher concentrations) on ICM development. A high glucose level was also inhibitory and increased dead-cell occurrence. The data suggest that insulin and glucose might interact and modulate blastocyst development as a function of their respective concentrations.
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Pregnancy in a diabetic mother involves a higher risk of early fetal loss and congenital malformations (1–5). Tight control of the diabetic state may decrease, although not completely normalize, the increased incidence of those dreadful complications (2,3). Many works performed in vivo or in vitro in animal models have addressed the physiopathology of diabetes-linked embryopathy. Most of these works have been devoted to the early postimplantation stages of pregnancy when organogenesis takes place (6–9). A few recent studies have drawn attention to the preimplantation stages of pregnancy, which could also be adversely affected by maternal diabetes (10–13). Our group recently reported a selective damage to the inner cell mass (ICM) development of day-5 blastocysts from diabetic rats (13). ICM has been shown to be more sensitive than the trophectoderm (TE) to many agents likely to adversely affect growth and development of the embryo (14). Because ICM is the primordial embryonic bud that will develop into all the embryonic structures, its selective damage could be significant for later stages of embryonic or fetal outcome (14,15).

High glucose (16,17) and high insulin levels have been shown to adversely affect the morphological development of preimplantation mouse embryos in vitro. On the other hand, receptors to insulin and insulinlike growth factor have been described on mouse morulae and blastocysts (18–20), and insulin may stimulate protein (20,21) and RNA (20,22) synthesis in vitro in preimplantation mouse blastocysts. Because of these recent in vivo and in vitro observations, we studied the interrelationship of glucose and insulin on the differential development of ICM and TE of rat blastocysts in vitro.

RESEARCH DESIGN AND METHODS

Female Wistar rats (70–90 days old) from the University of Louvain Breeding Center were mated overnight with males. The presence of a vaginal plug was designated as day 1 of pregnancy. The pregnant animals were killed in the morning (between 1000 and 1200) of day 5 or day 6, and the uterine horns were immediately recovered. Embryos were gently

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flushed from the uterine cavity with prewarmed Ham's F-10 medium (041-90088H, Gibco, Grand Island, NY), supplemented with L-glutamine (14.7 mg/L), penicillin (100 U/ml), and streptomycin (100 μg/ml), and observed on an inverted phase-contrast microscope. Blastocysts, characterized by the presence of a clear blastocoel, were individually transferred to incubating cells (Multidish, Nunclon Delta, 1-46485, Nunc Intermed, Roskilde, Denmark) in the same Ham's F-10 medium, containing 1 g/L bovine serum albumin (A 9647, Sigma, St. Louis, MO) and different concentrations of D-glucose and purified beef insulin (no. 680-3007, Gibco). Osmolarity of the incubation media was adjusted to 285–300 mosM with NaCl. Incubations lasted for 24 or 48 h at 37°C in an atmosphere of 5% CO_2/95% air. Glucose concentration in the incubating cells did not change significantly over that time due to the total glucose amount available per embryo (1 embryo/0.4 ml of medium).

At the end of the incubation time, embryos were recovered and processed through a differential cell-staining technique previously described (23), which allowed the separate counting of ICM and TE cells under a fluorescence microscope. Cellular death, occurring by apoptosis, was characterized by fragmented nuclei, appearing as fluorescent dots of unequal sizes inside a cell structure of irregular shape. Pyknotic debris, not included in a cell structure, was rarely observed and was not taken into account.

Embryos were randomly distributed in different experimental procedures run in parallel to avoid any litter effect on the development conditions. Results from at least four to six similar experiments were pooled to yield groups of 30–60 embryos suitable for statistical analysis. In such groups, any single pregnant rat contributed no more than 1 or 2 embryos. Comparisons were made between groups run in parallel to avoid slight variations observed between similar experiments performed at different times. Hence, control groups were repeated when necessary to ascertain the significance observed in experimental groups. The effects of glucose or insulin concentrations on embryo development were analyzed by nonparametric one-way analysis of variance (Kruskal-Wallis test). Differences between individual groups were submitted to the unpaired nonparametric Mann-Whitney test. Statistical significance of dead-cell numbers were obtained by the χ^2-test. Data in tables and figures are means ± SD.

RESULTS

Table 1 shows the differential cell counting of day-5 embryos cultured for 24 or 48 h in different concentrations of D-glucose (from 0 to 17 mM). At 24 h, there was no statistically significant effect of glucose levels on ICM cell number, although there was a tendency to lower values at 17 compared with 6 mM glucose ($P = 0.06$). On the other hand, glucose levels had a significant effect on TE cells due to lower development in the absence of glucose ($P < 0.01$). ICM ratios (% ICM in total cell number) were significantly different among the groups, decreasing with increasing glucose concentrations ($P < 0.001$). At 48 h, ICM cell number decreased significantly at a high glucose level ($P < 0.001$). Changing the medium after the first 24 h or decreasing glucose concentration from 17 to 6 mM during the second 24 h did not modify the lower ICM development in a high glucose concentration. ICM cell number was significantly lower at 48 h than at 24 h in 17 mM glucose ($P < 0.05$), suggesting a regression of ICM development (by cell death, for example) during the second 24 h of incubation. Glucose concentration had a minimal effect on TE development at 48 h, although a slight decrease occurred with 17 mM ($P = 0.05$). Changing the medium after 24 h was without effect on TE cell number. Whatever the concentration of glucose, TE cell number was higher at 48 than at 24 h ($P < 0.01$), attesting to continued development from 24 to 48 h. ICM ratio decreased significantly ($P < 0.001$) with increasing glucose concentration. This effect was mainly due to a larger reduction of ICM than of TE cells in 17 mM glucose.

The stimulatory effect of low insulin levels on day-5 blastocyst development was examined as follows. Blastocysts were incubated for 24 h in the presence of low insulin level (3 pM) at two glucose concentrations (1.1 and 6 mM). Figure 1 shows a slight, although significantly higher, ICM cell number both in 1.1 ($P < 0.05$) and 6 ($P < 0.01$) mM glucose, when insulin was present in the medium. TE cell number was

TABLE 1
Mean ± SE number of cells of day-5 embryos (*n*) after 24- or 48-h incubation in presence of different glucose concentrations

	Glucose concentration (mM)					
	0	1.1	6	17	17 + 17*	17 + 6*
24 h						
n	32	36	37	28		
ICM	16.3 ± 3.7	15.6 ± 3.7	16.2 ± 3.8	13.6 ± 4.7†		
TE	45.2 ± 6.9‡	51.7 ± 7.7	51.3 ± 7.5	50.1 ± 9.7		
Total	61.1 ± 9.9‡	67.3 ± 9.8	67.5 ± 10.2	63.7 ± 13.0		
% ICM	26.5 ± 3.6§	23.2 ± 4.7	23.8 ± 3.5	21.0 ± 5.7		
48 h						
n	42		45	32	34	28
ICM	18.4 ± 6.1		17.2 ± 4.1	11.4 ± 4.5§	10.7 ± 4.6	11.2 ± 4.9
TE	56.5 ± 13.2		61.4 ± 7.3	57.7 ± 8.4‖	57.9 ± 8.8	57.5 ± 8.6
Total	74.9 ± 16.3		78.6 ± 9.9	69.1 ± 10.3§	68.6 ± 9.7	68.6 ± 11.3
% ICM	24.5 ± 7.1		21.9 ± 3.4	16.3 ± 5.2§	15.6 ± 6.4	15.8 ± 5.2

ICM, inner cell mass; TE, trophectoderm. See RESULTS for variance analysis between all groups (Kruskal-Wallis tests).
*Embryos were incubated for 24 h in the presence of 17 mM glucose and further incubated for 24 h in the presence of 17 or 6 mM glucose.
†$P = 0.06$, ‡$P < 0.01$, §$P < 0.001$, ‖$P < 0.05$, vs. 6-mM group (determined by Mann-Whitney tests).

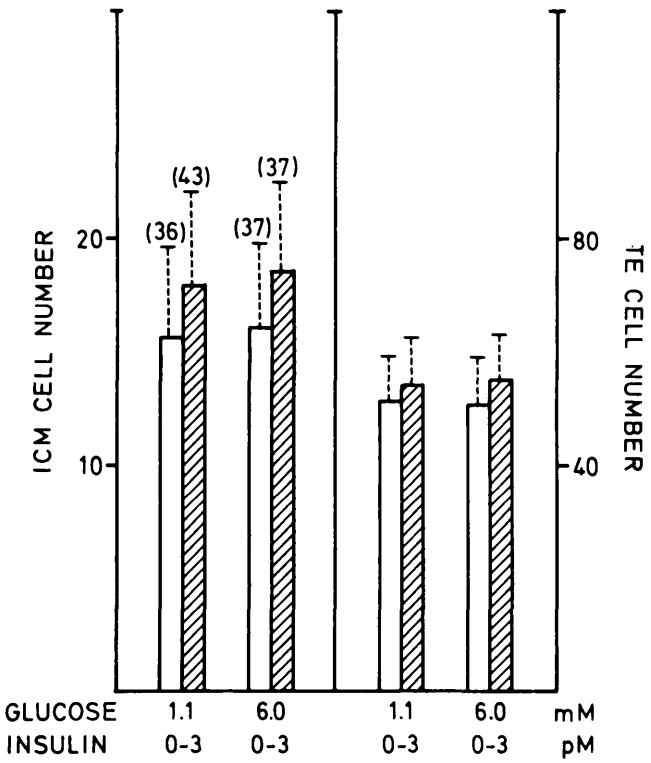

FIG. 1. Effect of low insulin concentrations on inner cell mass (ICM) and trophectoderm (TE) development of day-5 blastocysts incubated *for 24 h in different concentrations of* D*-glucose. Numbers of embryos are in parentheses. $P < 0.05$ and $P < 0.01$ for ICM in 1.1 and 6 mM glucose, respectively; $P < 0.01$ for TE in 6 mM glucose (by Mann-Whitney test between 0 [*open bars*] and 3 [*hatched bars*] pM insulin).

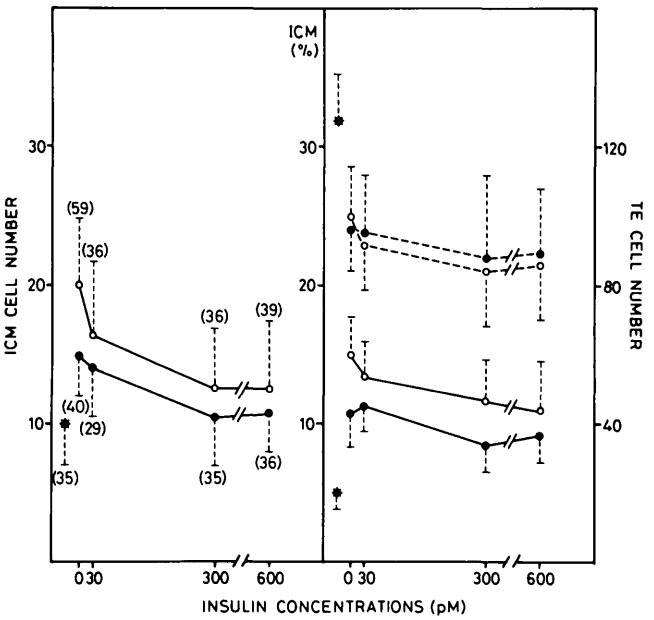

FIG. 2. Effect of increasing insulin concentrations on inner cell mass (ICM) and trophectoderm (TE) development and on ICM ratio (% ICM; *dashed lines*) of day-5 blastocysts incubated in presence of 6 mM glucose for 24 (●) or 48 (○) h. Numbers of embryos are in parentheses. $P < 0.001$ for insulin effect at both 24 and 48 h on both ICM and TE; $P < 0.05$, $P < 0.001$ on ICM ratios at 24 and 48 h, respectively (by Kruskal-Wallis tests). For further statistical analysis, see RESULTS. *Control embryos before incubation.

statistically higher ($P < 0.01$) in 6 mM glucose only. In the absence of glucose, this stimulatory effect of insulin was not observed (not shown).

We also examined the inhibitory effect of increasing insulin levels on day-5 blastocyst development. Blastocysts were incubated for 24 or 48 h in the presence of increasing levels of insulin and 6 mM glucose (Fig. 2). Both at 24 and 48 h, increasing insulin levels inhibited both ICM and TE development in a dose-dependent manner ($P < 0.001$). The inhibitory effect was relatively more important on the ICM than on the TE, as shown by the significant decrease in the ICM ratios with increasing insulin concentrations both at 24 ($P < 0.05$) and 48 ($P < 0.01$) h. The insulin-induced inhibition of development was not complete, however; indeed, a significant increase in ICM cell number was observed between 24 and 48 h both at 30 and 300 pM insulin ($P < 0.01$). At 600 pM insulin, the 24- to 48-h increase was not significant; however, the 48-h ICM cell number was significantly higher than the control number before incubation ($P < 0.01$). TE also continued to develop significantly between 24 and 48 h at insulin concentrations of 30 or 300 pM ($P < 0.001$) or at 600 pM ($P < 0.05$).

To test whether blastocysts could resume their development after insulin inhibition, embryos were cultured for 24 h in the presence of 600 pM insulin and further incubated for 24 h in a fresh medium with or without added insulin. Incubations were performed in the presence of 6 mM glucose throughout the 48 h. Table 2 shows that ICM development was inhibited after 48 h, whether the medium was changed after 24 h, if insulin was present throughout the whole period ($P < 0.001$). If insulin was removed after 24 h, ICM development at 48 h was still decreased compared with the control cultures without insulin ($P < 0.01$) but significantly less so compared with the cultures with insulin throughout the 48 h ($P < 0.01$).

Similarly TE development was partially inhibited by insulin, whether the medium was changed after 24 h, if insulin was present throughout the whole period ($P < 0.001$). However, the inhibition was less important when the medium was changed after 24 h ($P < 0.01$). Removing insulin after 24 h did not result in significantly higher cell development compared with changing the medium alone after 24 h ($P = 0.12$).

TABLE 2
Mean ± SE number of cells of day-5 embryos (n) after 48-h incubation in absence or presence (600 pM) of insulin during 1st 24 h only or during full 48 h (6 mM glucose)

Insulin concentration (pM)	Time (h)	n	Inner cell mass	Trophectoderm
0	48	59	19.7 ± 5.0	59.3 ± 11.4*
0 + 0†	24 + 24†	39	18.5 ± 5.4	63.2 ± 12.6‡§
600	48	39	12.3 ± 5.2*	45.0 ± 14.4*‖
600 + 600†	24 + 24†	35	12.0 ± 3.9¶	53.0 ± 12.6‡‖
600 + 0†	24 + 24†	47	15.5 ± 4.9¶	56.0 ± 12.1§

Statistical significance was determined with Mann-Whitney tests.
*$P < 0.001$, ‡$P < 0.01$, vs. inner cell mass.
†In these experiments, the medium was changed after 24 h and replaced by fresh medium with or without insulin at the indicated concentration.
§$P < 0.01$, ‖$P < 0.01$, vs. inner cell mass.
¶$P < 0.01$ vs. inner cell mass at 600 + 600.

The role of glucose in the insulin-induced inhibition of blastocyst development was studied by incubating embryos with increasing amounts of insulin for 48 h in the presence or the absence of 6 mM glucose. Figure 3 shows that, in the absence of glucose in the culture medium, insulin was without inhibitory effect on either ICM or TE development, whereas in the presence of glucose, both cell lineages were inhibited by insulin in a dose-dependent manner ($P < 0.001$). Figure 4 shows that, at a lower glucose level (1.1 mM), insulin was unable to inhibit ICM or TE development at 24 h, whereas the inhibitory effect on both cell lineages was observed in the presence of 6 mM glucose ($P < 0.001$).

On day 6, blastocysts were recovered and incubated for 24 h in the presence of insulin and 6 mM glucose. In the absence of insulin, total cell number increased significantly during the 24-h incubation time ($P < 0.001$). In the presence of insulin, the development was significantly decreased ($P < 0.001$; Table 3).

In the absence of glucose, the occurrence of dead cells in the ICM of day-5 blastocysts after 48-h culture was low, and insulin was without significant effect ($P = 0.12$; Table 4). In the presence of increasing levels of glucose, dead-cell number increased significantly up to 18% of all ICM cells ($P < 0.001$). This effect of glucose was also observed in the absence or presence of different concentrations of insulin ($P < 0.001$). However, in the presence of glucose (6 mM), increasing levels of insulin further increased the dead-cell number ($P < 0.001$).

DISCUSSION

Our previous in vivo studies showed that blastocyst development was impaired in day-5 or day-6 pregnant diabetic rats and that the ICM of the embryo was more affected by the maternal diabetic state than the TE (11–13). From in vitro studies, it was suggested that high glucose levels (16,17), serum from diabetic rats (16) or diabetic patients (24), or high insulin levels (25) could disturb the normal morphological evolution of mouse preimplantation embryos. In another recent study, rat morulae cultured in the presence of insulin achieved better development and a higher implantation rate after transfer but showed less-satisfactory development in later stages of pregnancy (26). Although little is known about the glucose or insulin levels of the uterine fluid in which the embryo will develop in the last stages of the preimplantation period, the maternal diabetic state will probably profoundly affect these levels.

In this study, we tried to approach some aspects of the potential glucose-insulin interaction by studying in vitro the effect of these factors on the development of the two cell lineages of blastocysts, the ICM and the TE. Blastocysts from day-5 pregnant rats were thus cultured in a composite medium (Ham's F-10), allowing a satisfactory cell growth for 24 h. Indeed, after that span of time, the number of both ICM and TE cells increased and reached levels close to those of blastocysts collected on day 6 (23; Table 3). As observed in vivo (23), ICM development in vitro was lower than that of TE, as evidenced by the decreased ICM ratio at the end of the incubation time. Cell growth was further increased

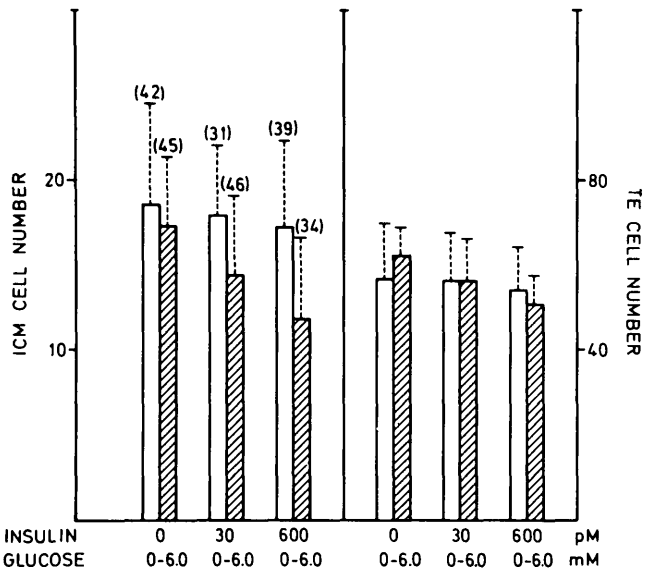

FIG. 3. Effect of increasing insulin concentrations on inner cell mass (ICM) and trophectoderm (TE) development of day-5 blastocysts incubated for 48 h in absence (*open bars*) or presence (*hatched bars*) of 6 mM glucose. Numbers of embryos are in parentheses. There was no statistically significant effect of insulin in absence of glucose; $P < 0.001$ in presence of glucose for both ICM and TE (by Kruskal-Wallis tests).

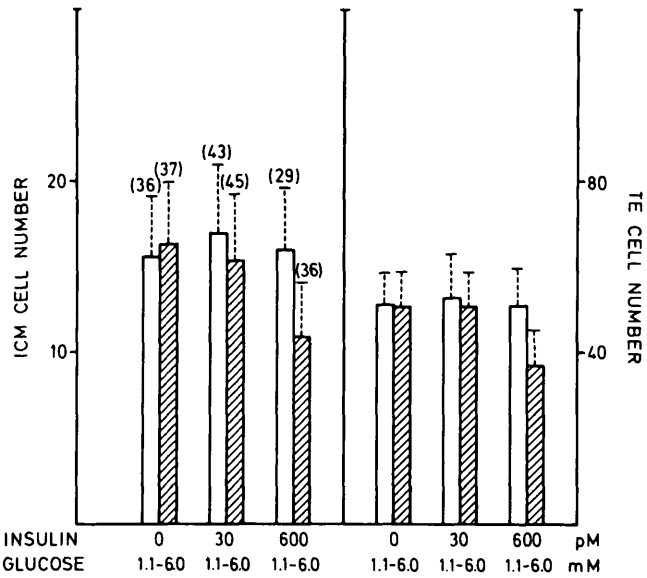

FIG. 4. Effect of increasing insulin concentrations on inner cell mass (ICM) and trophectoderm (TE) development of day-5 blastocysts incubated for 24 h in low (*open bars*) or high (*hatched bars*) glucose concentrations. Numbers of embryos are in parentheses. There was no statistically significant effect of insulin in presence of 1.1 mM glucose; $P < 0.001$ in presence of 6 mM glucose for both ICM and TE (by Kruskal-Wallis tests).

TABLE 3
Effect of insulin on day-6 embryos cultured for 24 h in 6 mM glucose

	Insulin (pM)			Preincubation control
	0	300	600	
Embryos (*n*)	32	30	33	25
Total cells (*n*)	98 ± 17	82 ± 11*	83 ± 17*	65 ± 11*

*$P < 0.001$ vs. 0 insulin.

TABLE 4
Number of dead cells in inner cell mass of embryos incubated for 48 h in presence of different concentrations of glucose and/or insulin

	Glucose (mM) + Insulin (pM)						
	0 + 0	0 + 30	0 + 600	6 + 0	6 + 30	6 + 600	17 + 0
Embryos (*n*)	61	47	44	193	109	91	120
Dead cells (*n*)	4	3	8	107	79	87	187
Dead cells (%)*	0.38	0.33	1.04	3.02	4.79	7.71	18.4

$P < 0.001$ for glucose effect in the absence of insulin; $P < 0.001$ for insulin effect in the presence of 6 mM glucose; $P = 0.12$ for insulin effect in the absence of glucose (all determined by χ^2-test).
*Percentage of total inner cell mass cell number.

after 48 h and reached, respectively, about two and three times the number of cells in the ICM and the TE of day-5 blastocysts before incubation. The rate of development from the 24- to 48-h incubation time was limited, however, compared with the first 24-h period. Although comparison with the in vivo situation is lacking (day-7 embryos have undergone implantation and cannot be recovered from flushing), day-6 embryos incubated for 24 h reached higher total cell number than day-5 embryos incubated for 48 h (see Table 3). Hence, embryo vitality was likely to be affected beyond the 24-h incubation time. Glucose levels in the culture medium were shown to influence blastocyst development. Although ICM development was not affected by the absence of glucose, TE was slightly, although significantly, less developed in a glucose-free medium. This observation is not unexpected because of the metabolic switch occurring in embryos from many species at the morula state, when glucose will be used increasingly as an energy source (27–31). However, this stimulatory effect of glucose (1.1 or 6 mM) was not observed for ICM. Moreover, a higher glucose level (17 mM), in the range of that encountered in the serum of diabetic animals, had a significant inhibitory effect on blastocyst development. This inhibitory effect was mainly observed on the ICM and much less so on the TE, further disclosing the divergent responses of the two embryonic cell lines to glucose. The deleterious effect of high glucose concentrations on preimplantation embryo development has been reported in other species (16,17). However, the glucose levels used in those studies were much higher than in this study and are not likely to occur in any physiological or pathological conditions. On the other hand in this study, we observed a more selective effect of glucose on ICM, i.e., on the embryonic bud; this selective damage might have untoward consequences on later stages of embryo development.

How glucose would impair ICM development is not known. In the hamster, it was shown that glucose could block cell development in the early stages of embryo growth, possibly by decreasing the activity of high-energy sources (32). Development of one-cell mouse embryos was similarly hampered by the presence of glucose in the culture medium (33). In other systems, such as endothelial cell cultures, high glucose levels could slow down the cell cycle traversal (34) and induce DNA damage (35). In this study, we observed a significant increase of dead cells occurring in 48-h cultures with high glucose levels. Also, the glucose effect on ICM development was irreversible after 24 h, and ICM cell number even decreased during the second 24-h period in the presence of high glucose, probably due to the increased occurrence of dead cells. This suggests that glucose, in the concentration range encountered in serum of diabetic animals, had a direct and permanent toxic effect on ICM cells.

Insulin was shown to improve embryo development at a low concentration (3 pM). This facilitating effect was more important in the ICM than in the TE. This observation fits with several reports in the mouse, showing that insulin receptors appeared on the preimplantation embryo at the morula stage and increased at the blastocyst stage (18,19). It has also been shown that insulin at such low levels could stimulate protein synthesis in the mouse compacted embryo (21). Although the blastocyst is probably unable to synthesize insulin (36), maternal insulin is present in the oviduct and is able to reach the ICM through the polar TE (20). However, similar evidence is still lacking regarding the rat. Also, insulin concentration in the oviductal or uterine fluids is unknown. Although the ratio between protein concentration in uterine fluid and in serum increases with decreasing molecular weight due to increased transfer (37), the concentration of maternal insulin in the genital tract may be much lower than the plasma level if the hormone transfer from serum to uterine fluid is hampered by high-molecular-weight protein association in the blood circulation.

When rat blastocysts were incubated in the presence of insulin levels within the 30- to 600-pM range of serum concentration, a dose-related inhibitory effect was observed mainly on ICM and, to a lesser extent, TE development. This inhibitory effect was almost maximal at a concentration of 300 pM, which is well within the physiological range of serum levels in the rat (unpublished observations). The EC_{50} of the inhibitory effect was <100 pM, which fits with the EC_{50} of insulin on glucose transport and is well below the binding saturation of the insulin receptors in adipose cells (38). It is thus likely that the inhibitory effect of insulin on blastocyst development was due to high-affinity binding to its own receptors and not to low-affinity cross-linking to the insulinlike growth factor receptors, although the latter appear on mouse morulae, together with the insulin receptor (19). The presence of insulin and insulinlike growth factor receptors on rat preimplanted embryos has not been reported, as far as we know.

How insulin, which is likely to exert its effect through specific binding to its own receptor, would inhibit blastocyst development is the subject of an interesting hypothesis. Insulin may regulate gene expression (39) and stimulate or inhibit protein synthesis (40,41) at all physiological concentrations. Insulin could also affect the metabolism of sub-

strates such as glucose. This hypothesis seems likely because we observed that insulin in the range of concentrations used in this study was without effect (neither stimulatory nor inhibitory) in the absence of glucose in the medium. Also, the inhibitory action of insulin was obtained only at a glucose concentration of 6 mM and not at the lower concentration of 1.1 mM. Hence, insulin could stimulate glucose uptake and/or its cellular metabolism, the result being either beneficial or detrimental to the cell development according to both insulin and glucose levels in the medium. Glucose transport into the preimplantation embryo is not fully understood, although a facilitating diffusion system has been described in other species (42). Insulin seems inactive on that system (42,43). However, insulin was shown to stimulate glucose utilization and incorporation in macromolecular fractions of mouse embryo (44). How such a stimulatory effect of insulin on glucose metabolism would eventually lead to growth inhibition is unknown. Whether the glucose-mediated insulin effect discussed here and the insulin-dependent glucose effect discussed previously are produced by similar or different mechanisms is also debatable. A direct action of insulin facilitated by glucose cannot be excluded (39).

The differential effects of insulin and glucose on the two blastocyst cell lineages reported herein would have been overlooked if morphological criteria such as overall aspect of blastomeres, blastocyst expansion, or hatching had been used as in other studies on preimplantation embryos (16,17,25). Indeed, insulin in the range of concentrations used in our study had no significant effect on blastocyst morphological development; 1.1 or 6 mM glucose had only a slight positive effect on such development (results not shown). Hence, profound impairment of embryo development may occur before the morphological aspect would be significantly changed.

The physiological or pathological implication of the data reported herein should await further knowledge about the physiologial concentrations of glucose and insulin in the maternal genital tract, which are under study. A likely hypothesis, based on a few reported data in several species (45,46), is that the glucose level might be severalfold lower in the genital tract than in the serum; insulin levels might also be significantly lower, although precise data are still lacking. This would lead to a physiological situation in which low levels of insulin and glucose would be stimulatory to embryonic development. In situations such as maternal diabetes, high glucose levels could become deleterious, mainly on ICM development, as observed previously in vivo in diabetic animals (13). Whether insulin treatment would improve the embryo development would then depend on the relative concentrations of glucose and insulin in the genital tract, the balance of which could be still inhibitory or become stimulatory. If transposed to the medical care of pregnant diabetic women, the above hypothesis would throw some light on unexpected failure of early treatment (2,3) and on a new approach of insulin administration, taking into account not only that the very early stage of embryo development is likely to suffer from the diabetic environment but also taking into account the potentially deleterious effect of high insulin levels in the presence of a high glucose concentration in the blastocyst environmental fluid.

In summary, we have shown that rat blastocyst development is impaired in vitro in the presence of high glucose levels (17 mM) and also in the presence of lower glucose levels (6 mM) and insulin in the physiological range of serum concentrations (30–600 pM). Low glucose (1.1- and 6-mM) and low insulin (3-pM) levels are stimulatory on embryo development. These data suggest that embryo development will be diversely influenced by the glucose-insulin balance in the maternal genital tract being stimulatory in physiological situations and probably inhibitory in situations such as diabetes mellitus.

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