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Research Article

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Factors Affecting the Response to Insulin in the Normal Subhuman Pregnant Primate

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ABSTRACT The concentrations of plasma glucose, free fatty acids, insulin, growth hormone, and placental prolactin in subhuman primate fetal and maternal plasma were examined following intravascular administration of insulin and glucagon to the fetus and mother. The neonatal plasma responses to these same stimuli were also examined.

Fetal plasma glucose concentrations were minimally altered by direct fetal insulin injections, whereas neonatal glucose levels declined with similar injections. In both instances, however, plasma free fatty acid levels declined following insulin. When the amount of insulin given the fetus was increased, fetal plasma glucose concentrations did decline. Combined intravascular insulin injections and infusions in the mother were associated with a disappearance of the initial maternal to fetal plasma glucose concentration gradient and a nearly parallel fall in both maternal and fetal plasma glucose levels. It was concluded that insulin was biologically active in the fetus. Obtunded fetal plasma glucose responses to direct fetal insulin administration may be a function of placental transfer of glucose from the maternal pool.

Maternal plasma placental prolactin and fetal plasma growth hormone levels were unchanged in the presence of sustained maternal and fetal hypoglycemia. However, neonatal plasma growth hormone levels did increase in response to hypoglycemia. The observed bidirectional placental barrier to transfer of radioisotopically labeled growth hormone indicated that fetal plasma growth hormone was solely of fetal origin. These data suggested further that a change in the growth hormone–releasing mechanism may occur from fetal to neonatal life.

Direct maternal intravascular glucagon administration led to augmentation in both maternal and fetal plasma insulin and glucose levels. Direct fetal glucagon injections enhanced both maternal and fetal plasma insulin levels. These simultaneous changes in both plasma pools were consistent with the demonstration of a bidirectional placental transfer of radioisotopically labeled glucagon. The role of endogenously produced glucagon in these studies remains to be clarified.

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INTRODUCTION

In a previous report from this laboratory (1), data pertinent to plasma glucose, insulin, and growth hormone interrelationships in the subhuman primate fetal-placental-maternal unit were detailed. It was determined that the fetal pancreatic islet during the late stages of gestation was not responsive to physiologic insulinogenic substances administered intravenously to the fetus; exogenously induced fetal hyperglycemia and hyperarginemia did not result in a change in fetal insulin or growth hormone plasma concentrations. However, tolbutamide administration to the fetus was associated with a prompt increase in fetal plasma insulin concentration. Interestingly, fetal plasma glucose concentrations were not altered in the presence of this induced fetal hyperinsulinemia. These observations, as well as other data reported from pregnant human primates (2–6), suggested that the fetal plasma glucose concentration was a direct function of maternal plasma glucose concentration as determined by the transplacental plasma glucose concentration gradient.

This present study was undertaken to investigate sequential changes in the concentrations of plasma glucose, growth hormone, free fatty acid, insulin, and placental lactogen in subhuman primate pregnancy after

the intravascular administration of insulin or glucagon to the fetus or mother. The experimental technique includes the cannulation of and blood sampling from interplacental fetal vessels in the pregnant rhesus monkey (*Macaca mulatta*) during the last trimester of pregnancy. In a further attempt to clarify any modifying influences of the placenta on fetal plasma responses, the bidirectional placental transfer of radioisotopically labeled growth hormone and glucagon and the neonatal plasma responses to the intravascular administration of insulin and glucagon were also examined.

METHODS

Surgical techniques. Pregnant rhesus monkeys (*Macaca mulatta*) with accurately known gestational ages maintained in the breeding colonies at the University of Pittsburgh School of Medicine were utilized. The length of pregnancy for the animals used in these studies ranged from 141 to 153 days; the gestational period for this species is approximately 164 days. The animals were maintained on biscuits (Purina Monkey Chow 25) and water ad lib., until they were fasted for the 12 hr before surgery. In animals in which radioactive iodine was used, 15 drops of Lugol's solution was orally administered 12 and 2 hr before the experiment.

The specific aspects of the medication, anesthesia, surgical technique, and postoperative care of the newborns have been previously reported (1). Briefly, under phencyclidine and halothane anesthesia, abdominal laparatomy was performed. Following an incision through the myometrium, decidua, and chorion, an interplacental fetal artery or vein was isolated and then cannulated with a silicone rubber T tube. This T tube permits both continuous flow through the vessel and sequential sampling of fetal blood while the fetus remains in the intact amniotic sac. Maternal blood was sampled through a cannula placed into the inferior vena cava from insertion at a saphenous vein.

Maternal blood samples were approximately 3.0 ml in volume; fetal blood samples did not exceed 1.2 ml. These aliquots of blood were replaced with equal volumes of dilute heparin–normal saline (5 U/ml). In experiments in which free fatty acids were measured, maternal and fetal cannulae patency was maintained by a constant infusion (0.02 ml/min) of nonheparinized lactated Ringer's solution.

At the end of the intrauterine experiment, the fetus was delivered and weighed, the umbilical vein was cannulated, and the newborn was then transferred to the nursery for supportive care after the intravenous administration of 250 mg of glucose. The neonate was orally administered 250 mg of glucose in water every 4 hr after delivery. Between 4 and 22 hr of life and after at least a 4 hr fast, experiments were performed on vigorous and clinically healthy neonates using the cannulated umbilical vein for blood sampling.

Placental transfer studies. The bidirectional placental transfer of growth hormone was studied in four animals. Purified human growth hormone (gift of the National Pituitary Agency, HS 503A) was isotopically labeled to specific activities of between 200 and 400 μCi/μg with either ^{125}I or ^{131}I respectively as described by Hunter and Greenwood (7). The iodination mixture was purified of damaged products and free radioactive iodine by passage through a G-75 Sephadex column eluted with Veronal buffer, pH 8.6. The purified eluate, diluted in rhesus monkey plasma, was subjected to hydrodynamic flow chromatoelectrophoresis (8) and to a double antibody precipitation technique to be described. On the days of isotope injections, greater than 93% of the iodinated growth hormone remained at the chromatograph origin.

In two of the animals, human growth hormone–^{131}I was injected into the maternal vena cava at the same time as human growth hormone–^{125}I was injected into the fetal vein. In two other animals, the injected materials were reversed with the mother receiving human growth hormone–^{125}I and the fetus growth hormone–^{131}I. The amount of radioactivity per injection did not exceed 75 μCi; the amount of labeled growth hormone injected did not exceed 0.2 μg. At intermittent intervals after the injections, blood samples were withdrawn simultaneously into heparinized syringes from the fetal and maternal circulations. The plasma was immediately separated and then kept at 4°C until analyzed in duplicate by a modification of the double antibody precipitation technique (9).

Plasma aliquots of 0.5 ml in 0.1 ml of 0.1 M ethylenediaminetetraacetic acid (EDTA) were incubated with 0.1 ml of guinea pig anti-growth hormone serum (final concentration, 1:600) for 24 hr. at 4°C. Then 0.1 ml of normal guinea pig plasma and 0.1 ml of rabbit anti-guinea pig globulin were added and the mixture was incubated for an additional 6 hr at 37°C. After centrifugation, the supernate was decanted, and the immunoprecipitate was washed twice with 0.1 ml of Veronal buffer, pH 8.6. The radioactivity in the immunoprecipitate was assayed in a gamma scintillation counter to a statistical error of less than 2%. All counts were corrected for background, decay, and volume. ^{131}I was counted in a window free of ^{125}I overflow. ^{125}I was then counted without overflow contamination after allowing ^{131}I to decay to insignificant levels.

With this technique, 88–92% of the radioactive counts in eluates of the labeled growth hormones used for injection were immunoprecipitable. When unlabeled human growth hormone and simian placental lactogen in concentrations up to 400 mμg/ml and 500 μg/ml respectively were added to rhesus monkey plasma containing radioisotopically labeled human growth hormone in vitro, the per cent immunoprecipitability of the tracer was not altered.

The unidirectional maternal to fetal and fetal to maternal placental transfer of glucagon was studied separately in two animals each. Purified glucagon (Lot 258-234B-167-1, The Lilly Research Laboratories, Indianapolis, Ind.) was isotopically labeled to specific activities of between 300 and 500 μCi/μg with ^{131}I (7). 25 μl of the iodination mixture was then placed on a 5 cm cellulose powder column (Whatman, CF11) prepared in a disposable capillary Pasteur pipette. After first washing the column with 2 ml of 0.1 M Veronal buffer (pH 8.6), the glucagon-^{131}I was subsequently eluted with three 0.5 ml washes of 0.1 M Veronal buffer containing 20% acetone and 0.25% human albumin (10). The purified eluate, diluted in rhesus monkey plasma, was subjected to hydrodynamic flow chromatoelectrophoresis (8). On the days of isotope injections, greater than 95% of the iodinated glucagon remained at the chromatogram origin.

In two animals, glucagon-^{131}I was injected into the maternal vena cava and intermittent blood samples were withdrawn simultaneously from the maternal and fetal circulations into heparinized syringes containing 2500 kallikrein inhibitor units (KIU) of Trasylol.[1] In two other animals, glucagon-^{131}I was injected into a fetal vessel and blood samples were similarly obtained simultaneously from the fetus and mother. The amount of radioactivity per injection did

[1] Purchased from F.B.A. Pharmaceuticals Inc., New York.

not exceed 0.6 mCi; the amount of labeled glucagon did not exceed 2 μg.

The plasma was immediately separated and duplicate 20-μl aliquots were then subjected to hydrodynamic flow chromatoelectrophoresis on Whatman 3MC chromatography paper for 2½ hr at 400 v. After the chromatograms dried, they were scanned in a 4π counter and the glucagon-^{131}I remaining at the chromatogram origin was separated from remaining radioisotopically labeled glucagon and free ^{131}I by cutting the strip. The radioactivity at the chromatogram origins was then separately determined in a gamma counter to a counting accuracy of greater than 98%.

The radioactive labeled glucagon was incubated in monkey plasma, in vitro, for 24 hr at room temperature. In separate control studies, progressive degradation of the radioactive labeled glucagon was observed over this period. The addition of 2500 KIU of Trasylol to the syringe in which the blood specimen was drawn completely protected the labeled glucagon from plasma-induced degradation (11).

Insulin administration studies. The fetal and simultaneous maternal plasma glucose, free fatty acids, immunoreactive insulin, growth hormone, and placental lactogen responses to single crystalline insulin (Iletin) injections, insulin injections and insulin infusions combined, and insulin infusions alone were examined. In six experiments insulin was intravascularly administered directly to the fetus; in four experiments insulin was infused intravenously to the mother. Blood samples were withdrawn simultaneously from the fetal and maternal circulations before and 15, 30, 40, and 60 min after the initial insulin injection or start of the insulin infusion. In five additional experiments, neonatal blood samples were obtained from the cannulated umbilical veins before and at 15, 30, 60, and 120 min after similar injections of insulin.

Glucagon administration studies. The fetal and simultaneous maternal and the neonatal plasma glucose, immunoreactive insulin, growth hormone, and placental lactogen responses to single glucagon[2] injections were examined. In five experiments, blood samples were withdrawn simultaneously from the fetal and maternal circulations before and 2, 5, 15, and 30 min after the glucagon injection. In two additional experiments, neonatal blood samples were obtained from the cannulated umbilical veins before and at 2, 5, 15, and 30 min following the glucagon injection.

The plasmas were separated immediately and analyzed in duplicate for glucose by a glucose oxidase method (Glucostat, Worthington Biochemical Corp.) and free fatty acids as described by Novak (12). Fetal and maternal insulin, fetal growth hormone, and maternal placental lactogen were measured by a modification (13) of the charcoal-coated radioimmunoassay method of Herbert, Kam-Seng, Gottlieb, and Bleicher (14). The specificity, sensitivity, and reproducibility of the insulin and growth hormone assays were previously reported (1, 15).

The placental lactogen content of maternal plasma was determined from a standard curve depicting the inhibition of the binding of human growth hormone–^{131}I to antiserum to human growth hormone by the addition of increasing amounts of unlabeled partially purified simian placental lactogen.[3] Experiments were performed to assess the immunologic similarity of the cross-reacting substance in simian fetal and maternal plasma and amniotic fluid with the simian placental lactogen used as a standard in the immunoassay. Plasma or amniotic fluid was assayed for simian placental lactogen at three or more dilutions and the slopes of the inhibition of the binding of human growth hormone–^{131}I to antiserum to human growth hormone was compared to that observed with unlabeled simian placental lactogen.

[2] Obtained from the Lilly Research Laboratory, Indianapolis, Ind., (Lot #258-234B-167-1) insulin immunoassay of this glucagon preparation revealed 5 mU of immunoreactive insulin per 1 mg of glucagon.

[3] This preparation was kindly supplied to us by Dr. Henry Friesen. It is anticipated that with further purification this standard may yield placental prolactin assays in monkey plasma which are quantitatively lower than those currently reported.

RESULTS

Control studies. Fig. 1 depicts the slopes of inhibition of the binding of human growth hormone–^{131}I to antiserum to human growth hormone after the addition to the incubation mixture of simian placental lactogen, simian maternal and fetal plasma, and simian amniotic fluid. Parellelism between the inhibition curves obtained from simian placental lactogen and maternal plasma and amniotic fluid provided evidence that these fluids contained placental lactogen or a substance immunologically similar to it. In our previous study (1) evidence was presented indicating immunologic similarity of the cross-reacting substance in simian fetal plasma with human growth hormone. Fig. 2 depicts a typical standard curve obtained and used in the immunoassay of placental lactogen in maternal plasma.

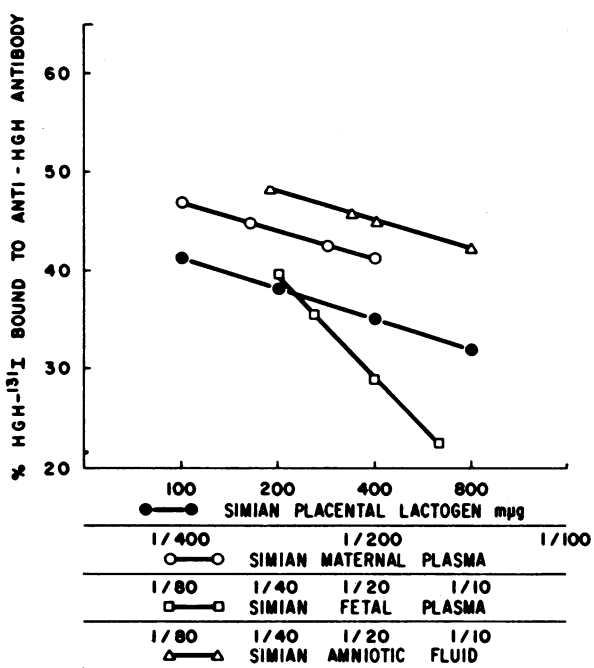

FIGURE 1 Inhibition of the binding of human growth hormone–^{131}I to antiserum to human growth hormone after the addition of either simian placental lactogen, simian maternal and fetal plasma, or simian amniotic fluid to the incubation mixtures.

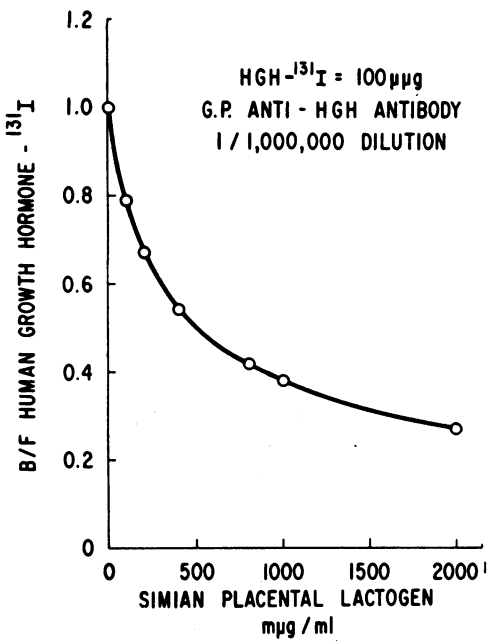

FIGURE 2 A typical standard curve obtained and used in the immunoassay of simian placental lactogen in maternal plasma.

Placental transfer studies. Fig. 3 depicts the maternal plasma disappearance curves of radioactive iodine–labeled growth hormone during 2 hr of sampling in two animals. Fig. 4 depicts the fetal plasma disappearance curves of the other radioactive and simultaneously injected iodine-labeled growth hormone over the same interval. (The curves in the two other animals not shown were similar to those depicted in these figures.) In no instance did the radioisotopically labeled growth hormone appear in the opposite circulation.

Figs. 5 and 6 depict the disappearance and appearance curves of radioactive labeled glucagon from two separate experiments during the 60 min of sampling. Similar results were obtained in the other two experiments. Glucagon crosses the placenta bidirectionally. The maternal to fetal exchange of glucagon (Fig. 5) was associated with steadily decreasing radioactive labeled glucagon concentration ratios which approached a count ratio of 1 : 1 at 60 min. When the fetus was the primary compartment, glucagon-^{131}I rapidly appeared in the maternal blood pool. At the termination of these experiments, the count ratio of fetal to maternal radioactive labeled glucagon approached 17 : 1 (Fig. 6).

Insulin administration studies. The fetal and maternal plasma glucose, free fatty acids, insulin, growth hormone, and placental lactogen responses to fetal insulin intravascular injections into the fetus and combined insulin intravascular injections and infusions into the fetus are recorded in Table I.

15 min after direct intravascular injections of insulin (0.09–0.21 U/kg) to the fetus 368F, 404F, 35F), fetal plasma insulin levels were 6- to 28-fold greater than the control level. Fetal plasma glucose levels were minimally reduced in the presence of this fetal hyperinsulinemia. Fetal plasma growth hormone, and maternal plasma glucose, insulin, and placental lactogen concentrations were not consistently altered. Insulin was also both injected and infused directly to the fetus (342F, 255F, 288F). At the termination of the insulin infusions, fetal plasma insulin concentrations of 21, 410, and 3500 mμg/ml, respectively, were achieved. In these experiments, fetal plasma glucose concentration did decrease 26% (342F), 17% (255F), and 61% (388F); fetal plasma growth hormone concentration and maternal plasma placental lactogen levels were not consistently altered. In one instance (388F), the maternal plasma glucose concentration was lowered. The maternal plasma insulin concentration in this experiment did change from 0.5 to 1.1 mμg/ml.

Fetal plasma free fatty acid concentrations were decreased subsequent to fetal insulin injections (404F) or insulin injections and infusions combined (342F, 388F). Maternal plasma free fatty acid levels were decreased in the only experiment (388F) in which a fall in maternal

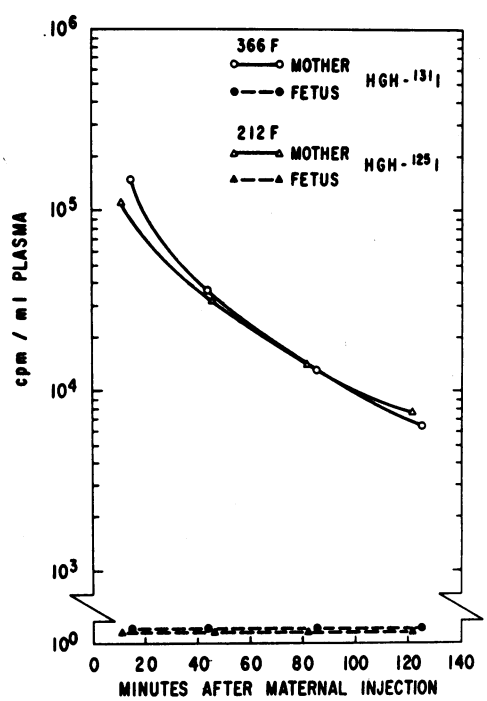

FIGURE 3 Relation between maternal and fetal radioactive iodine–labeled growth hormone after maternal injection. The fetus received simultaneously a different radioactive iodine–labeled growth hormone; see Fig. 4.

plasma glucose and a rise in maternal plasma insulin concentrations were also observed.

The fetal and maternal plasma glucose, free fatty acids, insulin, growth hormone, and placental lactogen responses to maternal insulin infusions (25F, 347F, 260F, and 381F) are recorded in Table II. The maternal plasma glucose concentration declined and reached a nadir 30–60 min after the initiation of the insulin infusion. The decline in maternal plasma glucose concentrations was associated with the disappearance of the initial maternal to fetal plasma glucose concentration gradient and a nearly parallel fall between the maternal and fetal plasma glucose concentrations. A reversal in the maternal to fetal glucose concentration gradient was observed at least once during each experiment. The fetal and maternal plasma concentrations of growth hormone and placental lactogen remained relatively constant in 25F, 260F, and 381F. In experiment 347F, the uterus contracted at irregular intervals for the last 45 min of the experiment during which time a rise in fetal plasma growth hormone concentration was observed. Both the maternal and fetal plasma free fatty acid concentrations fell during the maternal insulin infusion. The reduction in fetal free fatty acid levels was

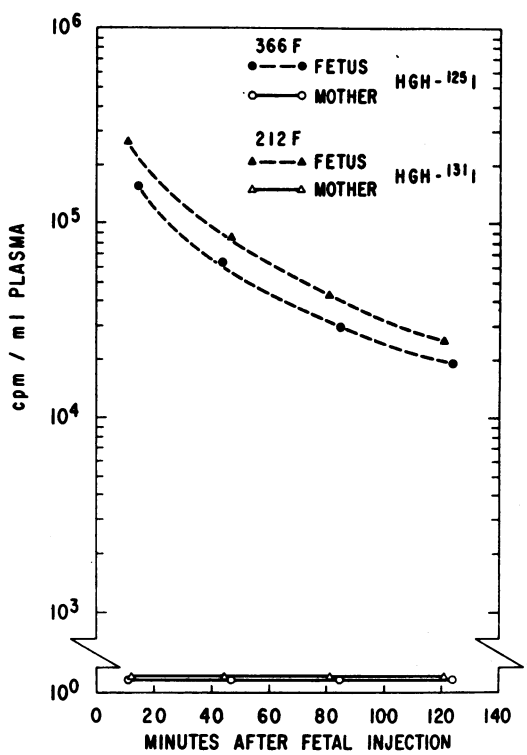

FIGURE 4 Relation between fetal and maternal radioactive iodine-labeled growth hormone after fetal injection. The mother received simultaneously a different radioactive iodine-labeled growth hormone; see Fig. 3.

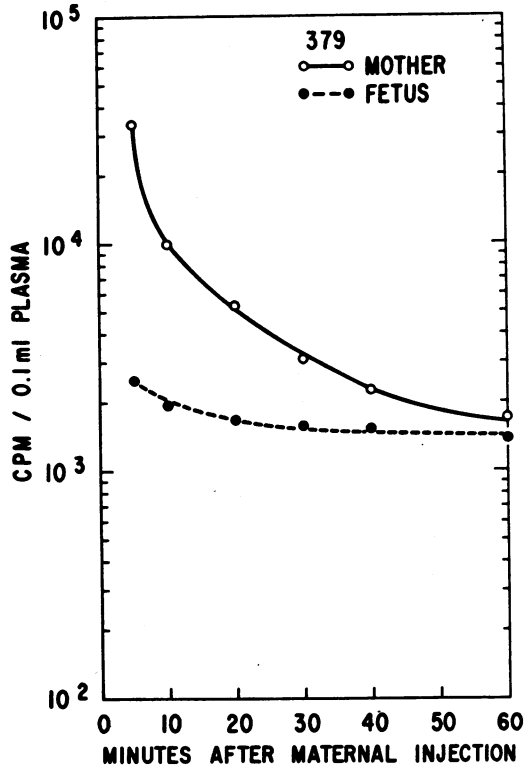

FIGURE 5 Relation between fetal and maternal radioactive iodine-labeled glucagon after maternal injection.

apparent 15 min after the initiation of the maternal insulin infusion at a time when fetal plasma insulin levels were only minimally increased.

The plasma glucose, free fatty acid, insulin, and growth hormone responses to the intravenous injection of insulin to the neonates delivered from these intrauterine experiments are detailed in Table III. The neonatal plasma concentration of glucose and free fatty acids fell after insulin injections, and an augmentation of plasma growth hormone concentration was observed. Plasma growth hormone concentrations in two of these neonates (25N, 260N) were not altered *in utero* in the presence of fetal hypoglycemia induced by maternal insulin infusions (Table II).

Glucagon administration studies. The maternal and fetal plasma glucose, immunoreactive insulin, growth hormone, and placental lactogen responses to intravascular glucagon injection administered to the fetus, mother, or neonate are recorded in Table IV. A prompt increase in fetal plasma glucose and insulin concentrations occurred following direct fetal glucagon injections (42F, 287F, 423F). During these fetal stimulation experiments, maternal plasma insulin concentrations also rose whereas augmentation in maternal plasma glucose levels was definitely observed only in the experiment (423F) in

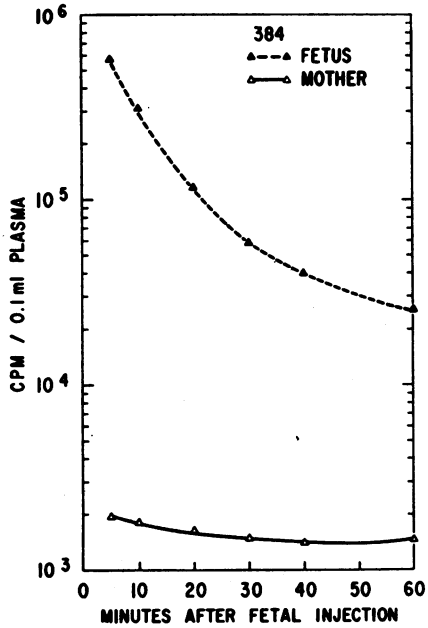

FIGURE 6 Relation between maternal and fetal radioactive iodine-labeled glucagon after fetal injection.

which the greatest increase in fetal plasma glucose concentration also occurred. Fetal plasma growth hormone and maternal plasma placental lactogen levels were not consistently altered.

Direct glucagon injection to the mother (371M, 344M) was associated with an augmentation of both maternal and fetal plasma glucose and insulin concentrations. Fetal plasma growth hormone and maternal plasma placental lactogen levels were not altered. Similar to the fetus and mother, direct glucagon injection to the neonate (423N, 380N) was also associated with a prompt rise in both neonatal plasma glucose and insulin concentrations.

DISCUSSION

Intravascular insulin injections to rhesus monkey neonates were associated with a gradual and persistent decline in plasma glucose concentrations. In contrast, plasma glucose levels were essentially unchanged after intravascular insulin injections administered directly to the intrauterine fetus. This apparent lack of sensitivity to insulin, *in utero,* may reflect specific characteristics of fetal tissue carbohydrate metabolism or may be a function of transplacental glucose transfer mechanisms. With regard to the former, published data utilizing nonprimate mammalian fetuses are contradictory (16, 17) and are of uncertain significance for primate fetuses. More pertinent are in vitro experiments on rhesus fetal skeletal muscle at 125 days gestation (18). In this tissue, insulin did enhance glucose uptake, lactate production, and the incorporation of ^{14}C from glucose-6-^{14}C into CO_2 and glycogen. Furthermore, in our experiments, when insulin is injected (404F, Table I) or injected and infused (324F, 388F, Table I) into the fetus, fetal plasma free fatty acids levels do decline independent of the maternal changes. Although the magnitude of the fetal fall, greater than 50% of base line values, may be influenced by concommitant transfer of free fatty acids from the maternal pool (19), it does provide additional evidence that insulin is biologically active in this primate fetus.

With regard to the latter possibility relating to placental transfer, although the available published data cannot distinguish whether glucose is transported across the primate placenta by either active transport or facilitated diffusion mechanisms (20–23), numerous studies have shown that fetal plasma concentrations are always lower than maternal concentrations, and that rapid transplacental equilibration occurs when maternal plasma glucose is raised (24–27). Following induced maternal hyperglycemia in humans in active labor, fetal scalp blood samples reveal rapid and parallel rises in fetal plasma glucose levels (5) with maintenance of the maternal to fetal glucose concentration gradient. In the present study, simian fetal plasma glucose also appear to change rapidly and be a function of maternal plasma glucose concentrations in the presence of maternal hyperglycemia as well as maternal hypoglycemia.

In the maternal insulin infusion experiments (Table II), the maternal-fetal plasma glucose concentration gradient initially disappeared and then, as the maternal plasma glucose level continued to fall, a nearly parallel fall in both maternal and fetal plasma glucose concentration was observed, with a reestablishment of the initial gradient as the mother's glucose level returned to base line. The dependency of fetal plasma glucose concentration on maternal plasma glucose concentration suggests that potential hypoglycemic effects of insulin administered to the fetus may be obscured by the rapid maternal to fetal transfer of glucose across the placenta. As seen in Table I, when the amount of insulin administered to the fetus was increased by combining insulin injections with insulin infusions, a definite lowering of fetal plasma glucose levels was observed. This decline is relatively small; it is essentially absent when lower exogenously induced insulin levels are achieved with single fetal injections alone.

The maximum rate of glucose disappearance from monkey neonatal blood over the 30 min period following the intravenous injection of a relatively large quantity of insulin (2.6 U/kg, 255N, Table III) is 1.5 mg/min. When lesser amounts of insulin are injected (404N, 35N, 25N, 260N, Table III) the rate of glucose disappearance from neonatal plasma is even

smaller (range 0.5–0.9 mg/min.) If we assume that the sensitivity to insulin is similar in both the fetus and the same animals as a neonate 4–6 hr later, it is possible to estimate the effect of glucose transferred across the placenta from mother to fetus on fetal plasma concentrations. Battaglia, Hellegers, Heller, and Bohrman have investigated glucose concentration gradients in the pregnant rhesus monkey (27). Using the arteriovenous glucose differences across the maternal and fetal surfaces in near-term gestations they approximated that 15 mg/min of glucose is consumed by the uterus and its contents under steady-state conditions. Howard and Krantz have obtained a placental transfer rate of 6 mg/min for term human placentas studied in an in vitro perfusion system (28) and a transfer rate of 10 mg/min glucose for ungulates has been reported (29). These rates considerably exceed the maximum rate of glucose disappearance from monkey neonatal plasma after intravenous insulin injection (Table III). Accordingly, it appears most likely that the placental transfer of glucose from

TABLE I

Effect of Intravascular Insulin Administration to the Fetus on the Plasma Glucose, Insulin, Free Fatty Acids, Growth Hormone, and Placental Lactogen Concentrations

Animal	Amount	Site	Plasma assay	Time (min) 0	15	30	45	60	Comment
368 F	0.03 U insulin injected at 0 min	Fetal artery	CHO*	34	33	33	29	29	145 day gestation,
			INS‡	1.3	8.2	2.7	2.3	2.0	mother 5.7 kg, female
			GH§	8	9	9	9	10	fetus 0.33 kg,
		Maternal vena cava	CHO	55	52	52	52	50	placenta 0.11 kg
			INS	2.2	2.6	2.6	2.4	2.1	
			SPL‖	225	225	200	225	225	
404 F	0.05 U insulin injected at 0 min	Fetal vein	CHO	44	44	41	39	39	142 day gestation,
			INS	0.4	11.1	3.0	2.6	1.6	mother 6.0 kg, female
			FFA¶	0.31	0.24	0.18	0.15	0.19	fetus 0.31 kg,
			GH	11	8	7	8	8	placenta 0.10 kg
		Maternal vena cava	CHO	57	58	56	53	55	
			INS	2.3	2.3	2.8	2.1	2.0	
			FFA	0.40	0.38	0.42	0.37	0.39	
			SPL	350	325	350	350	350	
35 F	0.10 U insulin injected at 0 min	Fetal artery	CHO	63	61	61	61	61	150 day gestation,
			INS	0.5	14.0	5.0	3.2	1.6	mother 7.8 kg, male
			GH	15	15	15	13	13	fetus 0.46 kg,
		Maternal vena cava	CHO	78	76	73	76	73	placenta 0.15 kg
			INS	2.6	2.5	2.3	2.3	2.5	
			SPL	325	275	275	300	325	
342 F	0.05 U insulin injected at 0 min, 0.01 U/min insulin infused for 15 min	Fetal artery	CHO	42	42	35	33	31	148 day gestation,
			INS	0.5	21.0	13.5	5.2	2.9	mother 5.5 kg, male
			FFA	0.41	0.29	0.25	0.25	0.21	fetus 0.36 kg,
			GH	5	5	6	6	8	placenta 0.11 kg
		Maternal vena cava	CHO	59	60	55	60	60	
			INS	0.9	1.1	1.1	0.9	1.0	
			FFA	1.12	1.12	1.28	1.16	1.15	
			SPL	250	275	250	250	250	
255 F	0.15 U insulin injected at 0 min, 0.05 U/min insulin infused for 15 min	Fetal artery	CHO	40	36	33	33	33	141 day gestation,
			INS	1.3	410	140	80	48	mother 5.5 kg, male
			GH	12	11	12	11	8	fetus 0.39 kg,
		Maternal vena cava	CHO	55	54	52	55	56	placenta 0.12 kg
			INS	1.9	1.9	2.1	2.2	2.1	
			SPL	200	200	200	225	200	
388 F	0.25 U insulin injected at 0 min, 0.10 U/min insulin infused for 15 min	Fetal artery	CHO	44	37	27	24	17	148 day gestation,
			INS	0.4	3500	580	270	180	mother 5.9 kg, female
			FFA	0.39	0.27	0.21	0.18	0.20	fetus 0.35 kg,
			GH	24	26	24	24	20	placenta 0.10 kg
		Maternal vena cava	CHO	52	50	46	45	41	
			INS	0.5	1.1	1.0	0.8	0.5	
			FFA	0.51	0.45	0.30	0.28	0.20	
			SPL	225	200	225	250	225	

* Plasma glucose mg/100 ml.
‡ Plasma insulin mμg/ml.
§ Plasma growth hormone mμg/ml.
‖ Plasma simian placental lactogen μg/ml.
¶ Plasma free fatty acids mEq/liter.

TABLE II

Effect of Intravenous Insulin Infusion to the Mother on the Plasma Glucose, Insulin, Free Fatty Acids, Placental Lactogen, and Growth Hormone Concentrations

Animal	Amount	Site	Plasma assay*	Time (min) 0	10	15	20	30	45	60	Comment
25 F	0.40 U/min insulin infused from 0 to 15 min	Maternal vena cava	CHO	54		29		24	17	23	153 day gestation, mother 7.3 kg, female fetus 0.45 kg, placenta 0.15 kg
			INS	2.2		525		235	118	66	
			SPL	300		300		300	325	325	
		Fetal vein	CHO	45		34		27	20	24	
			INS	1.5		8.4		7.9	5.4	4.2	
			GH	20		20		21	18	20	
347 F	Insulin infused at 0–10 min 0.30 U/min 10–20 min 0.58 U/min 20–30 min 0.82 U/min	Maternal vena cava	CHO	52		29		12	16	15	144 day gestation, mother 6.8 kg, male fetus 0.40 kg, placenta 0.14 kg; irregular uterine contractions beginning at 15 min
			INS	1.6		760		1320	460	155	
			FFA	0.63		0.40		0.35	0.27	0.15	
			SPL	325		350		325	300	300	
		Fetal artery	CHO	44		32		15	15	10	
			INS	0.8		1.4		3.0	5.0	2.6	
			FFA	0.48		0.31		0.29	0.23	0.19	
			GH	8		11		21	19	22	
260 F	Insulin infused at 0–10 min 0.30 U/min 10–20 min 0.58 U/min 20–30 min 0.82 U/min	Maternal vena cava	CHO	53	37		32	16	13	10	148 day gestation, mother 6.6 kg, female fetus 0.34 kg, placenta 0.11 kg
			INS	1.3	230		535	1520	520	160	
			FFA	0.77	0.39		0.27	0.22	0.19	0.22	
			SPL	325	325		325	325	325	300	
		Fetal artery	CHO	35	23		22	16	15	11	
			INS	0.9	1.4		1.6	2.8	4.2	3.6	
			FFA	0.52	0.46		0.33	0.26	0.28	0.30	
			GH	20	21		20	20	22	20	
381 F	Insulin infused at 0–10 min 0.30 U/min 10–20 min 0.59 U/min 20–30 min 0.82 U/min	Maternal vena cava	CHO	70	52		36	28		20	142 day gestation, mother 7.3 kg, female fetus 0.37 kg, placenta 0.12 kg
			INS	2.8	310		720	1360		140	
			SPL	250	250		250	250		250	
		Fetal vein	CHO	50	45		37	31	24	19	
			INS	1.0	1.3		3.6	7.2	5.9	2.8	
			GH	23	26		23	20	20	19	

* See footnote, Table I.

TABLE III

Effect of Intravascular Insulin Injection to the Neonate on the Plasma Glucose, Free Fatty Acids, Insulin, and Growth Hormone Concentrations

Animal	Amount	Site	Plasma assay*	Time (min) 0	15	30	60	120	Comment
404 N	0.05 U insulin injected at 0 min	Umbilical vein	CHO	47	37	20	15	12	5 hr of life, female 0.36 kg, fetal insulin injection
			INS	0.8	14.3	6.4	2.1	1.4	
			GH	10	18	32	26	22	
			FFA	1.25	0.59	0.48	0.48	0.52	
35 N	0.10 U insulin injected at 0 min	Umbilical vein	CHO	56	46	30	29	26	22 hr of life, male 0.46 kg, fetal insulin injection
			INS	0.6	16.0	3.9	1.8	0.8	
			GH	5	10	16	12	10	
255 N	1.0 U insulin injected at 0 min	Umbilical vein	CHO	73	56	34	29	12	20 hr of life, male 0.39 kg, fetal insulin injection and infusion
			INS	1.2	260.0	105.0	25.0	3.0	
			GH	8	14	19	17	16	
25 N	0.20 U insulin injected at 0 min	Umbilical vein	CHO	45	35	23	19	15	4 hr of life, female 0.45 kg, maternal insulin infusion
			INS	0.5	33.0	9.6	4.8	3.2	
			GH	5	5	13	26	24	
260 N	0.10 U insulin injected at 0 min	Umbilical vein	CHO	49	39	32	24	20	18 hr of life, female 0.34 kg, maternal insulin infusion
			INS	0.8	14.8	5.5	1.6	0.4	
			GH	8	8	14	28	26	
			FFA	1.80	1.30	0.50	0.39	0.27	

* See footnote Table I.

the readily available and relatively large maternal glucose pool accounts for the obtunded hypoglycemic responses to insulin in our fetal experiments.

Maternal and fetal plasma growth hormone and glucagon and maternal plasma placental prolactin could also affect the fetal plasma glucose responses to insulin administration. In our previous studies (1), the growth hormone–releasing mechanism was unresponsive in both the simian fetus and neonate when these animals were challenged by either induced hyperglycemia or induced hyperarginemia. Similarly, in the present study, insulin-induced maternal-fed hypglycemia was not associated with a change in the concentration of fetal plasma growth hormone. However, in contrast, an augmentation in neonatal plasma growth hormone levels was demonstrated in the presence of insulin-induced hypoglycemia. The absence of plasma growth hormone changes in the fetus could be explained if the fetal growth hormone levels were a function only of maternal growth hormone transferred across the placenta from the maternal pool. The placental barrier to the transfer of radioisotopically labeled human growth hormone demonstrated in this model, (Fig. 1), indicates that the immunoreactive growth hormone in simian fetal blood is probably solely of fetal origin. Laron, Pertzelman, Mannheimer, Goldman, and Guttman (30) have previously demonstrated that radioisotopically labeled and unlabeled growth hormone is not transferred from the maternal to fetal compartment in human subjects in active labor.

Another possibility to explain the fetal-neonatal difference in growth hormone responsiveness to hypoglycemia is that the growth hormone–releasing mechanism in the fetus is restrained by the same factor(s)

TABLE IV

Effect of Intravascular Glucagon Administration to the Fetus, Mother, or Neonate on the Plasma Glucose, Insulin, Growth Hormone, and Placental Lactogen Concentrations

Animal	Amount	Site	Plasma assay*	Time (min) 0	2	5	15	30	Comment
42 F	0.30 mg glucagon injected at 0 min	Fetal artery	CHO	49	61	66	56	47	143 day gestation,
			INS	0.5	2.4	1.6	0.9	0.5	mother 6.1 kg, female
			GH	16	16	19	22	17	fetus 0.33 kg, pla-
		Maternal vena cava	CHO	56	55	58	55	54	centa 0.11 kg
			INS	1.8	4.2	4.0	4.0	2.0	
			SPL	325	325	325	300	325	
287 F	0.30 mg glucagon injected at 0 min	Fetal vein	CHO	59	65	71	59	51	145 day gestation,
			INS	0.9	4.8	3.3	2.3	0.8	mother 6.5 kg, female
			GH	17	15	19	21	18	fetus 0.34 kg, pla-
		Maternal vena cava	CHO	69	72	73	68	65	centa 0.11 kg
			INS	1.7	2.6	5.8	4.1	1.9	
			SPL	275	250	250	250	250	
423 F	0.03 mg glucagon injected at 0 min	Fetal artery	CHO	36	40	41	65	84	153 day gestation,
			INS	1.0	4.4	3.2	3.2	2.0	mother 5.7 kg, male
			GH	24	24	29	26	25	fetus 0.41 kg, pla-
		Maternal vena cava	CHO	52	48	53	69	69	centa 0.14 kg
			INS	3.0	5.0	8.6	6.2	4.4	
			SPL	275	275	275	275	275	
371 M	0.19 mg glucagon injected at 0 min	Maternal vena cava	CHO	57	93	121	148	98	150 day gestation,
			INS	2.8	13.4	13.2	9.4	8.2	mother 6.4 kg, male
			SPL	300	300	325	300	300	fetus 0.41 kg, pla-
		Fetal vein	CHO	39	46	60	102	80	centa 0.11 kg
			INS	0.9	0.9	2.4	4.7	1.5	
			GH	27	27	20	21	24	
344 M	0.23 mg glucagon injected at 0 min	Maternal vena cava	CHO	40	40	54	80	67	146 day gestation,
			INS	3.6	15.4	8.6	4.2	3.3	mother 7.7 kg, female
			SPL	325	325	350	325	325	fetus 0.45 kg, pla-
		Fetal artery	CHO	32	36	47	58	30	centa 0.15 kg
			INS	0.5	0.9	1.5	3.2	3.8	
			GH	21	24	23	25	26	
423 N	0.03 mg glucagon injected at 0 min	Umbilical vein	CHO	19	23	28	35	24	15 hr of life, male
			INS	1.6	2.0	11.0	3.8	3.0	0.41 kg, fetal glucagon
			GH	17	20	19	13	20	injection
380 N	0.03 mg glucagon injected at 0 min	Umbilical vein	CHO	80	83	93	151	98	20 hr of life, female
			INS	2.0	7.2	3.4	2.4	2.2	0.35 kg, no fetal
			GH	10	12	11	15	13	experiment

* See footnote, Table I.

which inhibit maternal plasma growth hormone responses in late gestation. Mintz, Stock, Finster, and Taylor (31) and Katz, Grumbach, and Kaplan (32) have suggested that elevated levels of placental lactogen in the maternal circulation may inhibit the maternal growth hormone–releasing mechanisms. However, placental lactogen does not appear in the fetal circulation (Fig. 2) during the stage of gestation in which our experiments were performed.

Lastly, consideration should be given to the possibility that the growth hormone–releasing mechanism undergoes a change from fetal to neonatal life. We have previously demonstrated that simian fetal pancreatic islet cell responsiveness to glycemic stimulation is altered after birth (2) and Fischer and Odell (33) have also demonstrated a rapid change in pituitary thyrotrophin secretion shortly after delivery in the human. It remains to be proven wheher removal of the fetus from its major source of carbohydrate, its mother, initiates a growth hormone–adaptive mechanism to protect against the threat of glucose deprivation and (or) hypoglycemia.

Although levels of placental lactogen increase in pregnancy plasma throughout gestation (34), the role of this hormone and its relationship to gestational carbohydrate metabolism remain an enigma. Neither maternal hyperglycemia nor transient maternal hypoglycemia (35, 36) appears to affect the plasma concentration of placental lactogen in late gestation. In this study, no consistent maternal plasma placental lactogen changes were found in the presence of sustained maternal and fetal hypoglycemia. Similarly, in separate experiments, exogenously induced sustained fetal hyperglycemia also was not associated with altered maternal plasma concentrations of placental lactogen.[4] Although a role for the hormone in protecting a primary fetal claim on glucose has been suggested (37), we were unable to define a regulatory mechanism affecting the secretion of simian placental lactogen which was responsive to either an acute surfeit or paucity of glucose in the fetal-placental circulation.

The levels of both plasma glucose and insulin were augmented following the intravascular administration of glucagon to the fetus and the neonate (Table IV). In our previous study (1)), we demonstrated that induced fetal hyperglycemia was not associated with a change in base line fetal plasma insulin levels and a direct insulinogenic action of glucagon has been demonstrated in both human newborns (38) and isolated fetal pancreatic tissue (39). Accordingly, the hyperglycemic and insulinogenic actions of glucagon in simian fetuses appear to be separable and independent from each other. There are also data in the human adult that couples glucagon-induced glycogenolysis with antecedent hypoglycemia (40). Therefore, the possibility exists that endogenous glucagon influences fetal and maternal plasma glucose and insulin concentrations under these circumstances. The initial disappearance of the plasma glucose fetal-maternal concentration gradient in the maternal insulin infusion experiment (Table II) could be in response to glucagon-induced glycogenolysis of fetal-placental stores (41). Alternatively it may merely reflect a time delay in placental glucose transfer.

It does appear that if glucagon is involved in gestational carbohydrate metabolism, either a maternal or a fetal source could be bilaterally operational. The bidirectional placental transfer of radioisotopically labeled purified glucagon (Figs. 5 and 6) was relatively rapid compared to similar insulin experiments (1). Moreover, the administration of glucagon in one blood pool was associated with augmented plasma concentrations of insulin in the opposite one (Table IV). These simultaneous plasma changes in both blood pools are consistent with and confirmatory of the radioisotope-labeled transfer studies. Clarification of the role of endogenously produced glucagon will await the ability to specifically assay glucagon in fetal, maternal, and neonatal blood.

[4] Chez, R. A., and D. H. Mintz. Personal observations.

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