Human Fetal Insulin Metabolism Early in Gestation

Response to Acute Elevation of the Fetal Glucose Concentration and Placental Transfer of Human Insulin-I-131

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SUMMARY

Although insulin has been demonstrated in human fetal pancreas as early as thirteen weeks of gestation, the controls of insulin secretion in the human fetus and the magnitude of placental insulin transfer to the fetus are unknown. In pregnant women, scheduled for therapeutic abortions by abdominal hysterotomy at fifteen to twenty weeks of gestation, the fetal plasma insulin response to glucose infusion and insulin transfer across the placenta were studied as follows: (1) glucose was infused to eight fetuses in situ, and (2) insulin-I-131 was infused continuously for four to six hours to eight pregnant women via peripheral vein.

Insulin was measured radioimmunologically and, following the infusion studies, was precipitated quantitatively by a double antibody method. During fasting, no difference was observed between fetal and maternal plasma glucose or in insulin levels. Although glucose administered to the fetus raised the fetal plasma glucose concentration without changing the maternal level, both fetal and maternal plasma insulin concentrations were unchanged at five and ten minutes. Human insulin-I-131 was not transferred across the placenta and no sequestration of insulin-I-131 occurred in the placenta. Early in human gestation the fetal pancreas appears to be the major source of fetal insulin, and the fetal insulin secretion rate may be relatively unresponsive to acute changes in blood glucose concentration. The placenta acts as a barrier to human insulin-I-131 but does not appear to sequester and catabolize insulin-I-131, as was previously demonstrated in human pregnancies at term. DIABETES *18*:409-16, June, 1969.

Immediately after birth, infants of diabetic mothers dispose of glucose, either acquired transplacentally in utero[1] or administered postnatally as an acute intravenous load,[2] more rapidly than normal infants. Suppression of the normal postpartum increase of plasma free fatty acids also occurs in infants of diabetic mothers.[3] On the basis of an inverse relationship between maternal and neonatal glucose concentration, the rapid disposal of exogenous glucose loads, and the islet cell hypertrophy observed in infants of diabetic mothers, Pedersen[4] postulated hyperinsulinism stimulated by hyperglycemia in utero in the fetus of a diabetic pregnancy. Since insulin is present in the fetal pancreas early in gestation[5] and its physiological controls in utero are unknown, glucose was introduced to the human fetal circulation in situ to determine whether insulin secretion responds to an acute elevation of glucose concentration.

Placental insulin transfer has been studied in several mammalian species, including monkeys and man.[6-9] At term in man, Buse et al.[8] observed no insulin transfer; Gitlin,[9] however, detected low levels of human insulin-I-131 in newborn infants following administration of radioactive insulin to the mother. In the present study, steady-state insulin transfer from mother to fetus was evaluated during continuous infusion of human insulin-I-131 to the mother.

Presented in part at the seventy-eighth annual meeting of the American Pediatric Society on May 1, 1968.

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Studies of fetal blood pH and pCO_2 also were performed to validate the integrity of the fetal and placental circulation during these studies.

METHODS

Surgical procedures. Twenty-three pregnant women, ages sixteen to forty-one years, who required therapeutic abortion by abdominal hysterotomy participated in one of three studies. All patients were anesthetized by continuous administration of thiopental, succinyl choline, meperidine hydrochloride, nitrous oxide, and oxygen. During abdominal hysterotomy, fetal size was estimated approximately by palpation of the uterus, and the umbilical cord was exteriorized through a transverse incision in the lower uterine segment for injections or sampling. When sequential observations were obtained by repeated puncture of the fetal vessels, the umbilical cord was covered with a moist sponge between sampling procedures.

Maternal venous samples were obtained from an indwelling heparinized pediatric scalp vein set in one arm and from capillary samples by finger puncture. Radioactive materials were infused only to women who were to be sterilized during therapeutic abortion. For twenty-four hours preceding and seventy-two hours following the procedure, women who received insulin-I-131 were treated with saturated potassium iodide solution, fifteen drops by mouth three times daily. During the infusion study, insulin-I-131 solutions were administered continuously by peripheral vein in the arm contralateral to the maternal sampling site. When the fetus and placenta were delivered, they were examined carefully and the fetus was dissected. No abnormalities or deformities were evident from this visual examination.

Studies. (1) The fetal response to an acute glucose load was studied in eight pregnant women at sixteen to twenty weeks' gestation. The dose of 50 per cent glucose administered directly into the unoccluded umbilical vein varied from 0.6 to 2.0 ml., depending on the in situ estimation of fetal size. The mother and fetus were sampled simultaneously from the peripheral vein site and umbilical vein, respectively, both before glucose was administered and at five or ten minutes following the injection. Plasma insulin and glucose concentrations were determined in the fetal and maternal samples.

(2) Insulin transfer across the placenta was estimated in eight pregnant women between fifteen and sixteen weeks of gestation. Human insulin-I-131 was prepared by the Research Support Group, Abbott Radiopharmaceuticals, by a modification of the method of Hunter and Greenwood[10]; insulin-I-131 was separated from iodide-131 and degradation products by passage through Sephadex G-50 columns, and the solution of insulin-I-131 rendered bacteriologically sterile by millipore filtration. The specific activity of the labeled insulin varied from 160 to 309 mc./mg. (approximately 0.5 to 1.0 atoms of iodine per molecule). An acute prime injection of approximately 20 μc of human insulin-I-131 diluted in physiological saline with 0.5 per cent human serum albumin was injected over a two-minute period, followed by a continuous infusion at 20 μc/hr. One patient infused for 350 minutes received 134 μc, and the remaining patients who were infused for 250-270 minutes received less than 100 μc of insulin-I-131.

Maternal samples were obtained hourly and, at the time of delivery, simultaneously with samples of fetal umbilical vein blood and amniotic fluid. The plasma samples and a sample of infusate from the distal tip of the infusion tubing adjacent to the patient were processed immediately for quantitative antibody precipitation of insulin-I-131. The placenta and fetus were weighed, the fetus dissected and individual tissues weighed and the tissues homogenized for estimation of total radioactivity. In four studies plasma aliquots were also precipitated with TCA for estimation of the TCA soluble radioactivity. Aliquots of all the plasma samples were saved for insulin immunoassay and plasma glucose and free fatty acid determinations.

(3) The effects of abdominal hysterotomy alone and of the glucose injection upon fetal blood pH, pCO_2, and actual bicarbonate levels were studied in eight women and compared with the levels in maternal capillary samples. One of these women had twin fetuses and five of the patients were also subjects in the studies of glucose injection to the fetus.

Analyses. All blood samples were cooled on ice to 0° C. immediately, centrifuged, and separated at 0° C. Samples for quantitative precipitation of insulin-I-131 were processed completely on the day of the study. Aliquots for other analyses were stored at —20° C.

Plasma glucose concentration was estimated by a glucose oxidase method using the Worthington reagent; and plasma insulin concentration was estimated radioimmunologically following decay of the I-131 radioactivity by a modification of the method of Morgan and Lazarow.[11] The variance of the insulin standards expressed as the mean ± S.D. was as follows: 5± 0.9,

10 ± 1.1, and 40 ± 2.0 μU./ml. Plasma FFA was assayed following Dole extraction by the micro colorimetric method of Novak.[12]

Immediately following infusion studies, plasma insulin-I-131 was precipitated quantitatively by incubating 1 ml. aliquots of plasma with an excess of guinea pig anti-human insulin antiserum at 37° C. for thirty minutes, and precipitating the antigen-antibody complex with excess rabbit anti-guinea pig gamma globulin for twelve hours at 4° C. Ten per cent trichloracetic acid was added to an equal volume of other plasma aliquots to estimate TCA soluble radioactivity. Precipitates and supernatants from both the above procedures, and homogenates of fetal tissues from infusion studies were gamma counted for twenty minutes using the Nuclear Chicago Auto Gamma Scintillation System No. 4222.

Blood pH on maternal capillary and umbilical arterial and venous blood specimens was determined with Astrup micro pH equipment.* Blood pCO_2 was determined after equilibration of the samples at known high and low CO_2 tensions. Actual bicarbonate was calculated from the blood pH and its buffer characteristics using the modified Siggaard-Andersen nomograms.[13]

RESULTS

The effect of abdominal hysterotomy and glucose injection on blood pH and pCO_2. During the ten- to fifteen-minute period while the surgical procedure, injections, and sampling proceeded, the maternal pH was stable with a mild respiratory alkalosis caused by hyperventilation during anesthesia. The expected gradients for pH and pCO_2 were observed from maternal capillary blood to umbilical vein and umbilical artery (table 1). The mean levels of pH, relative to the maternal pH, were 0.07 units lower in the umbilical vein and 0.10-0.14 units lower in the umbilical artery. The pCO_2 was elevated 3.4 to 5.6 mm. Hg in the umbilical vein and 4.3 to 7.4 mm. Hg in the umbilical artery above the mean maternal pCO_2. The mean actual blood bicarbonate concentrations varied between 18 and 21 mEq./L., with no consistent significant differences between maternal and fetal levels.

Ten minutes following glucose injection, the mean fetal blood pH declined 0.05 ± 0.02 units (mean ± S.E.M.) from the preinjection level; pCO_2 had increased 2.25 ± 2.49 mm. Hg, and the actual bicarbonate concentration had declined 1.5 ± 1.4 mEq./L. Although these changes in parameters of acid-base balance were relatively small in the glucose-injected fetuses, measurements of pH and pCO_2 were not made in every glucose-treated fetus and greater alterations of pH and pCO_2 may have occurred in some of them.

The effect of glucose administration to the fetus in utero upon plasma insulin. At the time of delivery, the mean maternal and fetal plasma glucose concentrations were both 61 mg./100 ml. (table 2). Injection of glucose into the umbilical vein caused a statistically insignificant rise in the maternal plasma glucose concentration, but at five to ten minutes following the injection resulted in a variable increase in the fetal blood glucose concentration. In the two fetuses with the most prolonged gestation (twenty weeks), the glucose concentration rose only 1 mg./100 ml. and 10 mg./100 ml. The others had variable increments of 20 to 844 mg./100 ml. above the initial umbilical vein concentration. Assuming no increase in glucose uptake by the fetus, this increment in glucose concentration represented distribution in an apparent space greater than fetal weight in seven of the eight fetuses studied. In the one fetus with an increment of 844 mg./100 ml., the administered glucose was distributed in only 26 per cent of its weight. No studies of blood pH and

TABLE 1

Effect of abdominal hysterotomy and anesthesia on fetal pH and pCO_2 (mm. Hg)

Number of cases	Maternal capillary pH	Maternal capillary pCO_2	Umbilical vein pH	Umbilical vein pCO_2	Umbilical artery pH	Umbilical artery pCO_2
6	7.51 ±0.02*	23.7 ±1.8	7.44 ±0.02	29.3 ±1.8		
7	7.46 ±0.03	30.6 ±3.5			7.36 ±0.02	34.9 ±2.1
4	7.52 ±0.03	24.0 ±2.6	7.45 ±0.03	27.4 ±1.5	7.38 ±0.03	31.4 ±1.6

*Mean ± S.E.M.

TABLE 2

Effect of glucose administration via umbilical vein on plasma glucose and insulin concentrations

Sample (N = 8)	Time after injection (min.)	Plasma glucose (mg./100 ml.)	Plasma insulin (μU./ml.)
Maternal vein	0	61.3±3.4*	5.4±1.2
	5-10	65.4±4.7	4.9±1.0
Umbilical vein	0	61.4±3.4	6.0±0.8
	5-10	(53-892)†	5.3±0.4

*Mean ± S.E.M.
†Range of plasma glucose concentrations.

*Radiometer Co., Model AME 1.

TABLE 3
Effect of insulin-I-131 infusion on maternal plasma insulin glucose and FFA concentrations

		Period of infusion		
(N = 7)	Fast	180′	240′	270′
Insulin (μU./ml.)	6.4±0.7*	4.8±0.9	6.0±0.8	5.1±0.7
Glucose (mg./ 100 ml.)	74.7±3.1	70.3±1.9	74.6±5.1	71.0±8.0
FFA (μEq./L.)	1,161±108	700±72	938±104	1,082±257

*Mean ± S.E.M.

pCO_2 were done in this fetus.

Insulin was measurable in the plasma of every fetus studied at levels similar to those in the mothers. In one fetus who received no glucose, plasma insulin was detectable as early as eleven weeks of gestation.

Plasma insulin concentrations were measured in both umbilical artery and umbilical vein in six of the eight fetuses studied. The insulin concentrations in umbilical artery were the same as those in umbilical vein before and after stimulation with glucose (5.7 ± 0.8 μU./ml., mean ± S.E.M., n = 6). No rise in either the maternal or the fetal plasma insulin occurred, even when the glucose injection caused fetal hyperglycemia (table 2).*

Continuous human insulin-I-131 infusions

(1) *Effect upon maternal plasma insulin, glucose and free fatty acids.* During infusion of insulin-I-131, there was no measurable change in either plasma insulin or blood glucose concentration (table 3). The maternal plasma-free fatty acids declined from an initial mean concentration of 1,161 μEq./L. immediately after the initial venepunctures to 700 μEq./L. after 180 minutes of insulin-I-131 infusion. From 180 minutes until the time of delivery there was a gradual increase in plasma FFA to the fasting concentration.

(2) *Maternal plasma insulin radioactivity.* After 180 minutes of insulin-I-131 infusion, a relatively steady state had been established in which a slight but statistically insignificant increment in the radioactive insulin concentration of 7 ± 3.5 cpm./ml. (mean ± S.E.M.) occurred over a mean period of 52.4 minutes (determined on the seven patients who were infused 250-270 minutes). Assuming the 28 per cent volume of distribution observed by Vinnick and Freinkel in similar infusion studies on normal man,[15] the increment in insulin radioactivity in the women represented an accumulation of 2.2 per cent of the infused insulin. This contrasted with an accumulation of 119 ± 24 cpm./ml. during a mean 31.4 minute period at the end of the infusion studies, which included administration of anesthetic. This would represent an accumulation of 50 per cent of the infused radioactive insulin.

(3) *Transfer of human insulin-I-131 across the placenta.* The concentrations of plasma insulin radioactivity in eight women, in the plasma of seven of their fetuses, and in the amniotic fluid are documented in table 4. The total plasma radioactivity at the end of the infusions was 4,791 ± 475 cpm./ml. (mean ± S.E.M.) in the maternal vein plasma, and 834 ± 119 cpm./ml. in the fetal plasma. All maternal specimens were 1 ml. of plasma; two of the fetal specimens were 0.5 ml., the other five being 1 ml. of plasma; all precipitates and supernatants were counted for twenty minutes over a background of 60 cpm.

The proportion of radioactivity precipitated nonspecifically with the antibody to gamma-globulin was determined in separate analyses by incubating human plasma, human insulin-I-131, normal guinea pig serum as a carrier protein and rabbit antiguinea pig γ-globulin for twelve hours at 0° C. Over a wide range of radioactivity added to human plasma from 140 cpm./ml. to 140,000 cpm./ml., 0.64 per cent of the total counts were present in the precipitate after washing. This fraction of the total radioactivity present in plasma was subtracted to correct all estimates of plasma insulin-I-131 by antibody precipitation. Of the total radioactivity in maternal plasma, about one fifth was antibody precipitable insulin-I-131. None of the radioactivity detected in the fetal plasma was precipitable with antibody (table 4).

In four patients the plasma radioactivity was further fractionated into TCA soluble and TCA precipitable fractions (table 5). In the fetus 86 per cent of the radioactivity was TCA soluble "iodide," and 14 per cent TCA precipitable.

The total concentration of radioactivity in the placenta and tissues from seven fetuses is expressed as a per cent of the maternal plasma *total* radioactivity in table 6. In both placenta and fetal plasma the con-

*Since insulin transfer from mother to fetus was not observed in the present study, and since the glucose stimulus after acute injection to the fetus is difficult to quantify, further studies are in progress using continuous infusion of glucose to the mother for two hours before abdominal hysterotomy. Prolonged elevation of the fetal plasma glucose by this means causes only a minimal rise in the fetal plasma insulin concentration.[14]

TABLE 4
Concentration of human insulin-I-131 in mother and fetus (cpm./ml.)*

Patient	Maternal period of infusion 180′	240′	270′	Fetal specimen Umbilical vein	Amniotic
1	723 (240′)	924 (300′)	1,052 (342′)	0	NS†
2	699	745	814	NS	NS†
3	797	774	959	2	0
4	784	805	969	0	0
5	845	856	955	0	NS†
6	1,133	1,145	1,298	0	0
7	1,122	1,093	1,098	0	0
8	800	814	973	0	1
(N = 7)					
Mean ± S.E.M.	883 ±65	890 ±61	1,009 ±57	0	0

*Concentration of insulin radioactivity corrected for non-specific precipitation with antiguinea pig gamma-globulin (see text).
†NS = no sample.

centration was approximately 20 per cent of that in the maternal plasma. The other fetal tissues, except brain, contained a relatively constant concentration about 10 per cent of the maternal level, or 50 per cent of the fetal plasma concentration. Brain radioactivity remained at one third the level observed in other tissues. No sequestration of counts in placenta, liver, or kidney was observed.

(4) *Estimated insulin secretion rates.* Insulin secretion rates were calculated from the specific activity of the plasma insulin, and the rate of infusion of insulin-I-131 assuming an equal and constant, but unspecified, volume of distribution for both endogenous insulin and infused insulin-I-131, and assuming also the same fractional rates of disposal of each. The calculated rate of secretion was as follows:

$$S = \frac{I}{S.A.} - r$$

where S = endogenous secretion rate of insulin (mμg./hr.)
I = infusion rate of insulin-I-131 (cpm./hr.)
S.A. = specific activity of maternal plasma insulin (cpm./mμg.)
r = infusion rate of insulin-I-131 (mμg./hr.).

The infusion rate of insulin-I-131, r, was 1/50 — 1/13 of the calculated endogenous secretion rate; in the present studies the estimated insulin output was 23.2 ± 2.8 mμg./kg./hr. (mean ± S.E.M.) or 0.95 ± 0.20 units/day with a range of 0.5 to 2.0 units per day.

The fractional rate of disposal of insulin-I-131 was

TABLE 5
Fractionation of maternal and fetal plasma (per cent of total radioactivity)

Sample (N = 4)	Insulin per cent	Non-insulin TCA ppt* per cent	TCA soluble per cent
Maternal vein	20.1±1.7+	49.4±2.2	30.6±0.2
Umbilical vein	0	13.8±2.1	86.0±2.6

*Non-insulin TCA precipitable radioactivity calculated by subtracting plasma insulin-I-131 radioactivity from the TCA precipitable radioactivity.
†Mean ± S.E.M.

calculated from the steady state data as follows :

$$I = D = KCV$$

where I = the infusion rate of insulin-I-131 (cpm./min.)
D = the disposal rate of insulin-I-131 (cpm./min.)
K = the fractional disposal of insulin-I-131 ($min.^{-1}$)
C = the plasma concentration of insulin-I-131 (cpm./ml.)
V = the volume of distribution of insulin-I-131 (ml.).

Assuming a 28 per cent volume of distribution,[15] the fractional disposal of insulin-I-131 was 0.0068 $min.^{-1}$ and half time was 102 minutes.

DISCUSSION

The effect of anesthesia and glucose upon circulation. During the infusion of insulin-I-131 to the mother, a steady state was maintained between 180 and 240 minutes, although it was disturbed by the subsequent anesthesia and surgery. In the seven patients infused for 250-270 minutes, the observed accumulation of radioactivity in the maternal plasma during anesthesia

TABLE 6
Fetal tissue concentration of radioactivity following continuous infusions of insulin-I-131 (per cent total maternal plasma radioactivity)*

Tissue (N = 7)	Mean ± S.E.M. (per cent)
Plasma*	19.3±1.3
Placenta	21.6±3.1
Umbilical cord	11.8±2.1
Carcass	11.3±1.6
Lung	10.4±1.3
Liver	8.4±1.1
Kidney	10.5±1.5
Heart	8.0±1.1
G.I. tract	9.7±1.2
Brain	3.2±0.4

*Total plasma radioactivity in maternal plasma (cpm./ml.) = 100 per cent. Total radioactivity in fetal plasma (cpm./ml.) or tissues (cpm./gm.) compared as follows: $\frac{\text{fetal tissue radioactivity}}{\text{maternal total plasma radioactivity}} \times 100$.

could represent a decline in the apparent volume of distribution of insulin-I-131 of 11.7 per cent, a decline of 58 per cent in the fractional disposal of insulin-I-131, or a combination of both. Since thiopental tends to decrease splanchnic blood flow and to raise total peripheral resistance, a change in volume is likely. Specifically, Habif, Papper et al.[16] have demonstrated that both renal and hepatic blood flow may decline markedly during anesthesia with thiopental. These alterations in hepatic and renal circulation would reduce the availability of insulin-I-131 to these two major sites of its sequestration,[17] destruction and elimination.[18,19] Thus a combined effect of anesthesia on both volume of distribution and fractional rate of destruction is likely on the basis of circulatory alterations alone. A direct effect of anesthesia and metabolic degradation of insulin-I-131 is also possible.

Since the anesthetic agents also could have reduced placental and fetal perfusion, blood pH and pCO_2 were measured directly in the fetus to evaluate the integrity of the feto-placental circulatory complex. Validation of placental circulation was important in the transfer studies, particularly since the distribution of insulin-I-131 in the mother appeared to change during anesthesia. At the time of the initial sample, the fetus demonstrated the expected gradient in pH and carbon dioxide tension.[20-21] Since the gradient for the rapidly diffusible carbon dioxide was a moderate one and the plasma pH relatively stable, the placenta probably was well perfused by both the maternal and fetal circulation.

Effect of glucose administration to the fetus. Basal insulin levels in the mother and fetus were studied to determine whether the fetal pancreas secretes insulin. Since the fetus and mother had similar insulin concentrations in the apparent absence of insulin transfer from the maternal circulation, the fetal pancreas probably not only manufactures insulin[5] but also secretes insulin as early as eleven weeks of gestation.

One other reason for performing these studies was to determine whether the fetus responds to alterations in glucose concentrations with increased insulin output. The plasma insulin levels in the fetus at less than twenty weeks gestation were, like the mothers', in the low normal fasting range. Rapid injection of glucose had no effect on the fetal insulin concentration. Since insulin transfer was not demonstrated at this gestational age, a more appropriate method of studying the problem may be to infuse the mother continuously, before delivery, with glucose to achieve more prolonged stimulation without disrupting the fetal circulation.*

Biological activity and catabolism of insulin-I-131. In order to assess the biological effect of the infused insulin-I-131 on metabolism, plasma glucose and free fatty acid concentrations were measured. Since the calculated increase in insulin concentration during the steady state would be less than 0.5 μU./ml. at the rate of insulin-I-131 disposal observed, the stable plasma glucose and insulin concentrations were expected. Although plasma free fatty acid levels are more sensitive than blood glucose to changes in the plasma insulin concentration, the prime injection of insulin-I-131 would raise plasma insulin less than 1 μU./ml. before distribution beyond the blood volume and less than 0.5 μU./ml. after distribution in the extracellular fluid. The decline and subsequent rise in plasma free fatty acid concentrations were, therefore, unexpected.

Izzo et al. have demonstrated a direct relationship between the degree of insulin iodination and the attenuation of biological activity.[22] Since unlabeled and iodine-labeled insulin share competitively catabolic pathways in both perfused tissues and intact animals,[23,24] the fractional disappearance of insulin-I-131 was estimated to assess attenuation of the biological disposal by iodination.

In the present study of pregnant women, the mean disposal rate of iodinated insulin was similar to that observed by Vinnick and Freinkel after insulin-I-131 was infused continuously to fasting man,[15] but much slower than the initial rate observed for radioiodinated insulin after acute intravenous injections to human subjects.[25,26] This more rapid disappearance following acute injection may reflect not only insulin-I-131 degradation, but also continued mixing of the protein molecule in its virtual volume of distribution. Recently Stern et al.,[27] using an immunological method to identify labeled insulin in plasma, measured the "insulin delivery rate" in normal man following a single acute injection of insulin-I-131. Since the insulin specific-activity time curve consisted of more than one exponential, the "insulin irreversible loss rate" from the plasma pool was estimated from the injected dose of radioiodinated insulin and the area under the specific activity time curve.

The fractional irreversible removal of insulin from the plasma pool following its distribution in the mul-

*Prolonged elevation of the fetal plasma glucose by continuous infusion of glucose to the mother raises only minimally the fetal plasma insulin concentration early in gestation.[14]

ticompartmental system that Stern describes can be calculated assuming the apparent virtual volume of distribution of insulin-I-131 described by Vinnick.[15] The mean fractional removal of insulin estimated from Stern's data is the same as that estimated by Vinnick following continuous infusion of insulin-I-131 to normal adult males (approximately 0.0085 vs 0.0089 $min.^{-1}$). The slightly lower estimated basal insulin disposal rates in the present study may reflect a greater volume of distribution of insulin-I-131 in pregnant women, an attenuated disposal of insulin during pregnancy, or possibly moderate attenuation of insulin-I-131 disposal by its iodination.

Transfer of insulin-I-131. Although unconjugated nonprotein-bound-fat-soluble steroid hormones diffuse rapidly across the placenta,[28,29] the transfer of water-soluble proteins is highly selective.[30-32] When a family of proteins is transferred, the efficiency of a type-specific mechanism may increase with gestational age.[32] The two previous studies of human maternal to fetal insulin transfer were conducted at term. Gitlin[9] detected materno-fetal transfer of small quantities of human insulin-I-131, but Buse[8] detected no transfer of bovine insulin-I-131. Both studies, however, used nonspecific methods for detection of insulin-I-131 and although some transfer of insulin-I-131 may occur at term, the maximum insulin transfer observed could have supplied only a small fraction of the fetal plasma insulin pool.

In the present study at sixteen weeks' gestation, no significant materno-fetal insulin-I-131 transfer was detected, even though a high level of activity was maintained in maternal plasma for several hours. Iodine may have occupied and altered a site on insulin specifically required for insulin transfer, but data concerning this hypothesis were not obtained in this study.

Following infusion of insulin-I-131 to the mother, TCA precipitable-I-131 radioactivity was detected in fetal plasma. This fraction may have represented insulin-I-131 partially degraded in maternal tissues or placenta and transferred to the fetal circulation, plus a small quantity of iodide-131 bound to fetal plasma protein.[8] An alternate hypothesis could be transfer of insulin-I-131 to the fetus, with rapid degradation in the fetus. If this hypothesis were true, sequestration of I-131 in the fetal liver and kidney may have been expected, since these are the usual major sites of insulin-I-131 sequestration in vivo.[17] Sequestration of tissue-bound iodide-131 in the fetus could be inferred from differences between total tissue radioactivity and the calculated concentration of soluble iodide in the tissues. Assuming that the TCA soluble radioactivity observed in the fetus was iodide,[8] the mean soluble iodide concentration in fetal tissue may be calculated from the plasma concentration of iodide-131 and the apparent fetal iodide space.

Although there are no measurements of the iodide space in utero, Fisher et al.[33] have demonstrated decreasing distribution of injected iodide-131 during human development, from a maximum observed value in the newborns to much lower levels in the adult. Three hundred minutes after injection of sodium iodide-131 to newborns, the mean iodide space was 53.1 per cent of the body weight.[34] Based on these measurements, the expected mean soluble iodide-131 concentration in fetal tissues would be at least 45.7 per cent of the total fetal plasma radioactivity or 8.8 per cent of the total maternal plasma radioactivity. Comparison of this calculated value with the observed total radioactivity in homogenized tissues (table 6) confirms that there was no apparent sequestration of radioactivity in liver or kidney. Since there was no accumulation of radioactivity in either of these organs, placental transfer and subsequent degradation of insulin-I-131 seems unlikely.

Buse et al.[8] demonstrated, however, a marked in vivo sequestration and in vitro catabolism of TCA precipitable radioactivity in the human placenta following insulin-I-131 infusion of the mother at term. During those studies, they also measured both the placental iodide space after injection of sodium iodide-131 to the mother and the volume of the maternal plasma retained in placenta after injection of albumin-I-131. When the total radioactivity in the placenta at term was corrected for iodide-131 and for TCA precipitable radioactivity confined to the maternal vascular pool of the placenta, the TCA precipitable activity was 2.5 to 3.5 times higher in the placenta than in maternal plasma. In contrast, when the data obtained at sixteen weeks' gestation were corrected in the same manner, the calculated concentration of total radoiactivity in the placenta is 22.1 per cent of the total activity in the maternal plasma. This compares with the level of 21.6 per cent observed at sixteen weeks of gestation (table 6). Thus, there is no accumulation of TCA precipitable radioactivity in the placenta at this early gestational age. The early human placenta appears to act only as a barrier to insulin-I-131 transfer without the capacity, developed by term, to sequester and catabolize insulin.

ACKNOWLEDGMENT

This work was supported by research grants from the Cleveland Diabetes Fund; the Association for the

Aid of Crippled Children; the Sigrid Juselius Foundation; and the U.S. Public Health Service, National Institutes of Health (#5-TI-AM-5356 and #1-SO1-FR-05507).

Sterile human insulin-I-131 solutions were prepared by B. J. Green, the Research Support Group, Abbott Radiopharmaceuticals, North Chicago, Illinois.

The authors are grateful to Dr. Bengt Meyer for his assistance and to the operating room nursing staff at the Women's Hospital and at the Boije Hospital, Helsinki, Finland.

REFERENCES

[1] McCann, M. L., Chen, C. H., Katigbak, E. B., Kotchen, J. M., Likly B. F., and Schwartz, R.: Effects of fructose on hypoglucosemia in infants of diabetic mothers. New Eng. J. Med. *275*:1, 1966.

[2] Baird, J. D., and Farquhar, J. W.: Insulin secreting capacity in newborn infants of normal and diabetic women. Lancet *1*:71, 1962.

[3] Chen, C. H., Adam, P. A. J., Laskowski, D. E., McCann, M. L., and Schwartz, R.: The plasma free fatty acid composition and blood glucose of normal and diabetic pregnant women and of their newborns. Pediatrics *36*:843, 1965.

[4] Pedersen, J., Bojsen-Møller, B., and Poulsen, H.: Blood sugar in newborn infants of diabetic mothers. Acta Edocr. *15*:33, 1954.

[5] Grillo, T. A. I., and Shima, K.: Insulin content and enzyme histochemistry of the human foetal pancreatic islet. J. Endocr. *36*:151, 1966.

[6] Goodner, C. J., and Freinkel, N. F.: Studies on the permeability of rat placenta to I-131-insulin. Diabetes *10*:383, 1961.

[7] Josimovich, J. B., and Knobil, E.: Placental transfer of I-131-insulin in the rhesus monkey. Amer. J. Physiol. *200*:471, 1961.

[8] Buse, M. G., Roberts, W. J., and Buse, J.: The role of the human placenta in the transfer and metabolism of insulin. J. Clin. Invest. *41*:29, 1962.

[9] Gitlin, D., Kumate, J., and Morales, C.: On the transport of insulin across the human placenta. Pediatrics *35*:65, 1965.

[10] Hunter, W. M., and Greenwood, F. C.: Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature *194*:495, 1962.

[11] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody system. Plasma insulin levels of normal, subdiabetic, and diabetic rats. Diabetes *12*:115, 1963.

[12] Novak, M.: Colorimetric ultramicro method for the determination of free fatty acids. J. Lipid Res. *6*:431, 1965.

[13] Siggaard-Andersen, O.: The pH-log pCO_2 blood acid base nomogram revised. Scand. J. Clin. Lab. Invest. *14*:598, 1962.

[14] Obenshain, S. S., Raivio, K., Teramo, K., King, K. C., Adam, P. A. J., Räihä, N., and Schwartz, R.: Response of the human fetal pancreas to prolonged glucose stimulation. Personal communication.

[15] Vinnick, L., and Freinkel, N.: Basal insulin turnover. Clin. Res. *14*:290, 1966.

[16] Habif, D. V., Papper, E. M., Fitzpatrick, H. F., Lowrance, P., McC-Smythe, C., and Bradley, S. E.: The renal and hepatic blood flow, glomerular filtration rate, and urinary output of electrolytes during cyclopropane, ether, and thiopental anaesthesia, operation, and the immediate post operative period. Surgery *30*:241, 1951.

[17] Elgee, N. J., Williams, R. H., and Lee, N. D.: Distribution and degradation studies with insulin-I-131. J. Clin. Invest. *33*:1252, 1954.

[18] Samols, E., and Ryder, J. A.: Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and exogenous insulin. J. Clin. Invest. *40*:2092, 1961.

[19] Chamberlain, N. J., and Stimmler, L.: The renal handling of insulin. J. Clin. Invest. *46*:911, 1967.

[20] Dawes, G. S.: Fetal blood gas tensions and pH. *In* Fetal and Neonatal Physiology. Chicago, Year Book Medical Publishers, Inc., p. 106, 1968.

[21] *Ibid*: The fetal circulation. p. 91.

[22] Izzo, I. J., Roncone, A., Izzo, M. J., and Bale, W. F.: Relationship between degree of iodination of insulin and its biological, electrophoretic, and immunochemical properties. J. Biol. Chem. *239*:3749, 1964.

[23] Mortimore, G. E., Tietze, F., and Stetten, D., Jr.: Metabolism of insulin-I-131; Studies in isolated perfused rat liver and hind limb preparations. Diabetes *8*:307, 1959.

[24] Wick, A. N., and Drury, D.: The effects of superimposed native insulin on disposal of iodoinsulin in the body. Proc. Soc. Exp. Biol. Med. *97*:514, 1958.

[25] Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., and Newerley, K.: Insulin-I-131 metabolism in human subjects: demonsrration of insulin-binding globulin in the circulation of insulin treated subjects. J. Clin. Invest. *35*:170, 1956.

[26] Welsh, G. W., Henley, E. D., Williams, R. H., and Cox, R. W.: Insulin-I-131 metabolism in man. Plasma binding, distribution, and degradation. Amer. J. Med. *21*:324, 1956.

[27] Stern, M. P., Farquhar, J. W., Silvers, A., and Reaven, G. M.: Insulin delivery rate into plasma in normal and diabetic subjects. J. Clin. Invest. *47*:1947, 1968.

[28] Migeon, C. J., Bertrand, J., and Wall, P. E.: Physiological disposition of 4-C-14-cortisol during late pregnancy. J. Clin. Invest. *36*:1350, 1957.

[29] Sandberg, A. A., and Slaunwhite, W. R., Jr.: Transcortin: a corticosteroid-binding protein of plasma. II. Levels in various conditions and the effects of estrogens. J. Clin. Invest. *38*:1290, 1959.

[30] Gitlin, D., Kumate, J., Urrusti, J., and Morales, C.: The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. J. Clin. Invest. *43*:1938, 1964.

[31] Bangham, D. R., Hobbs, K. R., and Terry, R. J.: Selective placental transfer of serum proteins in the rhesus. Lancet *2*:351, 1958.

[32] Dancis, J., Lind, J., Oratz, M., Smolens, J., and Vara, P.: Fetal transfer of proteins in human gestation. Amer. J. Obstet. Gynec. *82*:167, 1961.

[33] Oddie, T. H., Fisher, D. A., Wait, J. C., and Newton, B.: Radioiodide space in human subjects without edema. J. Clin. Endocr. *24*:54, 1964.

[34] Fisher, D. A., Oddie, T. H., and Burroughs, J. C.: Thyroidal radioiodine uptake rate measurement in infants. Amer. J. Dis. Child. *103*:738, 1962.