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THE EFFECTS OF PREGNANCY ON METABOLIC FUELS*

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When food is withheld, peripheral caloric needs are increasingly fulfilled by the metabolism of fat. Eventually, full adaptation to fat supervenes. Expenditure of carbons from sources other than fat is then cut down completely and restricted to an irreducible proportion of the oxidative demands of the nervous system (Cahill, 1970). Comparable parsimony is not possible in pregnancy; some additional losses of maternal fuels are always operative (Freinkel, 1965, 1969). For example, net gain in total fetal weight during days 17 to 19 of gestation in the rat is not significantly different whether the previously well-fed mother is fasted completely during this interval (and loses an average of 35.4 g *in toto*) or given unrestricted access to food (and gains an average of 28.1 g) (Herrera *et al.*, 1969*b*). In other words, the growing feti extract fuels unremittingly from the mother regardless of the maternal opportunities for replenishment.

What fuels are employed? Transplacental passage of esterified and free fatty acids (FFA) differs in various species; ketones seem to cross the placenta readily in all (Dawes, 1968). However, building blocks other than 2-carbon fragments are required by the fetus for anabolic esterification of fatty acids, glycogenesis, and synthesis of protein. Such building blocks as glucose cross the placenta freely, and amino acids are actually concentrated in the fetus against a transplacental gradient (Dawes, 1968). Maternal amino acids are further depleted by heightened urinary losses (Christensen *et al.*, 1957; Zinneman *et al.*, 1967).

Thus, the fasting gravid animal cannot conserve her glucose nor her amino acids like the non-gravid. Instead, she must divert extrauterine structures to the utilization of fat at a more rapid rate, and effect more efficient mechanisms for gluconeogenesis and conservation of nitrogen.

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Today we should like to summarize the investigations into maternal fuel homeostasis that we have conducted (Freinkel, 1969; Freinkel *et al.*, 1970; Herrera *et al.*, 1969*a*, *b*; Knopp *et al.*, 1970*a*, *b*; Metzger *et al.*, 1969, 1970; Shambaugh *et al.*, 1971) since we last reviewed our experiences before this Congress (Freinkel, 1965). We have employed the pregnant rat for our recent efforts, since this model permits more heroic manipulations than the gravid human.

Relationships between fuels and insulin in the rat

How do the relationships between plasma fuels and insulin in the rat simulate the human? Table I summarizes measurements secured on day 19 of the 22-day rat gestation period (Freinkel *et al.*, 1970; Herrera *et al.*, 1969*b*). Animals had been fed or fasted for 48 hours, and blood was drawn from the aorta under Nembutal anesthesia (40 mg/kg). In confirmation of Scow *et al.* (1964), we observed lower plasma glucose and higher plasma FFA in pregnant than in age-matched virgin females under both conditions. Moreover, the fast for roughly 10% of the total gestation period results in near hypoglycemia in the mother.

TABLE I

*Effect of pregnancy on circulating fuels and insulin in fed and 48-hour fasted rats**

	Day of gestation		P
	0	19	
Fed rats			
Plasma			
Glucose (mg/100 ml)	108.6± 4.6 (10)	80.9± 2.8 (10)	<0.001
FFA (μEq./l)	310 ±15 (7)	516 ±75 (7)	<0.02
Ketones (μEq./l)	285 ±37 (7)	145 ±11 (7)	<0.01
Insulin (μU/ml)	20.1± 2.5 (10)	34.2± 4.4 (10)	<0.02
Insulin/glucose	18.3± 2.1	41.8± 4.5	<0.001
48-hour fasted rats			
Plasma			
Glucose	96.5± 1.0 (11)	51.3± 3.2 (8)	<0.001
FFA	443 ± 21 (7)	739 ± 59 (7)	<0.001
Ketones	1781 ±157 (7)	6473 ±549 (7)	<0.001
Insulin	9.7± 1.1 (11)	12.8± 2.1 (8)	NS
Insulin/glucose	9.9± 0.9	23.9± 3.5	<0.001

* Plasma specimens were secured on day 19 of gestation from pregnant rats which had been fasted for the preceding 48 hours (*i.e.* days 17–19 of gestation) or fed to the time of sacrifice. Age-matched virgin animals ('0 gestation') were processed concurrently in the same fashion as described in the text. *P* denotes significance of differences between mean ± SEM values for virgin and pregnant animals. NS = not significant.

In the fed 19-day pregnant rat, plasma insulin is higher than in the non-gravid animals. After a 48-hour fast, the levels in the pregnant rats no longer differ significantly from those in the virgins, although the significant increases in the ratio of plasma insulin/glucose persist. Plasma ketones disclose some important differences between fed and fasted states. Despite higher levels of FFA, the fed pregnant rats maintain lower plasma ketones than control animals. This would suggest that something is driving lipolysis which is not offset by the

'extra' insulin, although the insu' acts within the liver to div e FFA to products other than ketones. On the other han in the fasted animals, hep ketogenesis appears less restrained than in non-gravid rat .

Does the 'extra' insulin repr sent potent hormone or son immunologically cross-reactive material of lesser activity? An indirect answer was sough by administering crystalline insulin (10 U/kg), or equivalent volume of saline, to fed and 48-hour fasted 19-day pregnant and age-matched virgin rats (Knopp *et al.*, 1970*b*). Acute hypoglycemic effects of the insulin were derived by subtracting the blood sugar values obtained after insulin injection from those observed after administration of saline and 'handling' (Figure 1). Clearly, acute hypoglycemic responses to insulin are smaller in gravid than in virgin animals under fed as well as fasted conditions. In other words, the presence of the conceptus appears to antagonize the hypoglycemic actions of insulin in the rat, as in the human (Burt, 1956). The insulin-resistant effects of gestation are exaggerated when dietary deprivation is added.

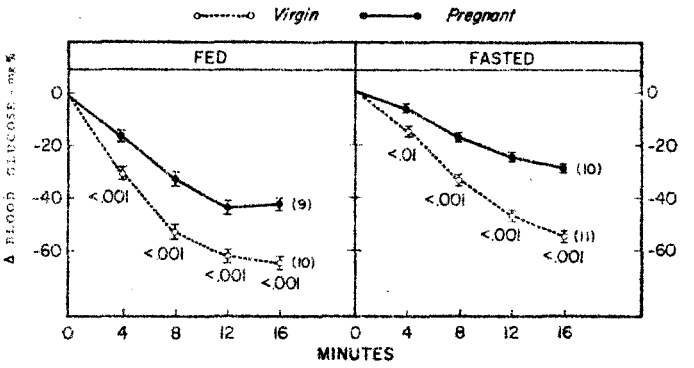

Fig. 1. Effect of intravenous insulin on blood glucose in the rat. Values represent mean ± SEM of differences between blood glucose values following the administration of insulin (10 U/kg) or equivalent volumes of saline to fed or 48-hour fasted pregnant and age-matched virgin animals. Mean ± SEM and significant differences (pregnant *vs.* virgin) are indicated at each time point. ( ) indicates the number of animals in each group.

Metabolism of adipose tissue

'Steady-state' values for FFA and glycerol in lumbar fat from fed and 48-hour fasted 19-day pregnant rats are shown in Table II and contrasted to similar measurements in virgin females. In both situations, adipose tissue from pregnant animals contains more FFA per mg tissue protein. Thus, the elevations of plasma FFA which are seen during late pregnancy even in the fed state must reflect an increased availability of FFA from adipose tissue stores. In theory, the latter could occur via: (*a*) a primary decrease in esterification due to lower plasma glucose or diminished effectiveness of insulin upon glucose utilization, or (*b*) a primary increase in lipolysis due to increased availability of a lipolytic agent(s?) or lack of response to the restraining effect of insulin upon triglyceride lipase. We found that increased lipolysis is implicated (Freinkel *et al.*, 1970; Knopp *et al.*, 1970*a*). During prolonged incubation *in vitro*, segments of lumbar fat from fed pregnant rats produce more glycerol and FFA than comparable pieces of adipose tissue from age-matched virgin animals. The augmented breakdown of preformed glycerides is independent of the availability of glucose; and *in vitro* responsiveness to the antilipolytic and the lipogenic effects of small amounts of insulin is preserved. In the presence of albumin and glucose, esterificatio is enhanced. In other words, isolated fat from the fed 19-day pregnant rat behaves as if sub cted to a continuing primary

TABLE II

*Effect of pregnancy on adipose tissue content of FFA and glycerol**

	Pregnant	Virgin	P
	(μmoles/mg tissue protein)		
Fed rats			
FFA	0.258±0.038 (6)	0.147±0.023 (8)	<.02
Glycerol	0.012±0.001 (6)	0.011±0.002 (6)	NS
Fasted rats			
FFA	0.560±0.036 (12)	0.222±0.014 (8)	<.001
Glycerol	0.036±0.005 (8)	0.020±0.002 (12)	<.01

* Segments of lumbar fat were rapidly excised from 19-day pregnant and age-matched virgin rats which had been given uninterrupted access to food ('fed') or deprived of food for 48 hours ('fasted') prior to sacrifice. Mean ± SEM values are listed above; ( ) denotes number of animals; P indicates significance of differences between values in pregnant and virgin rats; NS = not significant.

stimulation of lipolysis. Since esterification and responsiveness to insulin are unimpaired, net loss of fatty acids during the fed state *in vivo* may be minimized by the greater intake of food and the increased availability of insulin. However, heightened turnover of adipose stores is always present so that the pregnant animal appears better poised to mobilize preformed fat when exogenous nutrients are withheld.

Glucose production in the liver

The livers of the 19-day pregnant rats are heavier (Beaton *et al.*, 1954; Bokelmann and Schringer, 1932; Campbell and Kosterlitz, 1949; Freinkel, 1969; Freinkel *et al.*, 1970; Herrera *et al.*, 1969*b*; Poo *et al.*, 1939; Scow *et al.*, 1964) and contain more and larger liver cells (Campbell *et al.*, 1953; Campbell and Kosterlitz, 1949; Freinkel, 1969; Freinkel *et al.*, 1970; Herrera *et al.*, 1969*b*). Theoretically, these anatomical features might confer greater biosynthetic performance. To document gluconeogenic efficiency in the intact animals, virgin and pregnant rats were injected with 1 mmole of pyruvate 3-^{14}C intravenously and blood was sampled and analysed for labeled glucose during the subsequent 30 minutes (Freinkel, 1969; Freinkel *et al.*, 1970; Herrera *et al.*, 1969*b*). Values for specific activity of plasma glucose are shown in Figure 2. In fed 19-day pregnant rats these values were not significantly different than in virgin animals, indicating that the 'extra' insulin may be offsetting potential biosynthetic advantages. On the other hand, following fast for 24 or 48 hours, the rates of glucose renewal from labeled pyruvate were significantly greater in the gravid animals.

To document that these effects were not due to simple differences in the splanchnic delivery of precursor, liver perfusion was employed (Metzger *et al.*, 1969, 1970). Livers from 19-day pregnant and age-matched virgin animals which had been fasted for 24 hours were perfused with Krebs-Ringer bicarbonate containing 3% 'fat-free' albumin and sufficient bovine red blood cells to provide a hematocrit of 20–22%; 17 mM L-alanine was employed as the gluconeogenic substrate. Preliminary perfusion was instituted for 25 minutes to remove endogenous metabolites, hormones and other extracellular variables; then definitive perfusion with fresh medium was carried out for another 60 minutes.

Figure 3 depicts net production of glucose from 17 mM L-alanine during the latter perfusion. At all intervals, more glucose was formed per μmole hepatic DNA by the livers of the pregnant rats. Uniformly labeled ^{14}C-L-alanine was employed to document disposition of alanine carbons in greater detail.

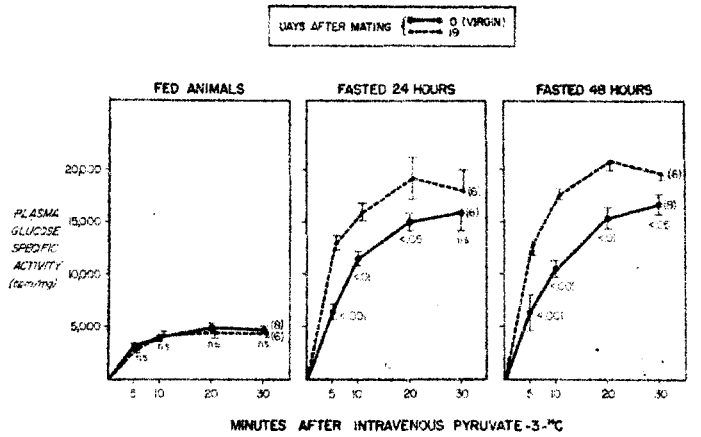

Fig. 2. Effect of pregnancy on glucose renewal from pyruvate-3-^{14}C in the rat. Fed or fasted 19-day pregnant and age-matched virgin animals received 1 mmole ^{14}C-pyruvate at time 0. Mean ± SEM and significant differences (pregnant *vs.* virgin) are denoted at each time point. NS = not significant. ( ) denotes the number of animals in each group.

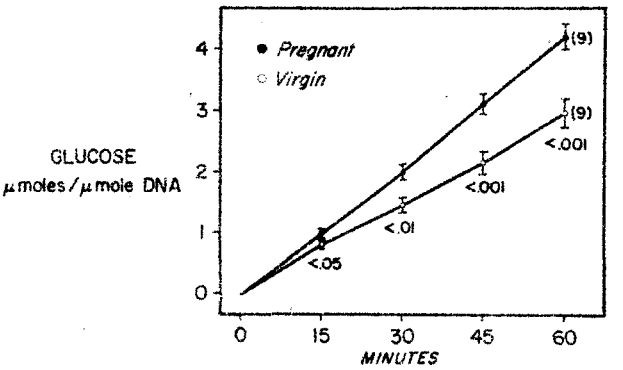

Fig. 3. Effect of pregnancy on net glucose production during 60 min. perfusion of rat liver with 17 mM L-alanine. Livers were obtained from 24-hour fasted 19-day pregnant and virgin littermate rats. ( ) indicates the number of perfusions in each group. Mean ± SEM and significant differences (pregnant *vs.* virgin) are indicated at each time point.

Values for the net utilization of ^{14}C-alanine and its incorporation into labeled glucose, glutamate, lactate and glyceride-glycerol per μmole hepatic DNA during 60 minutes' perfusion of livers from pregnant animals are shown in Figure 4 as percentages of the values observed under similar conditions with livers from virgin rats – *i.e.*, % control. By this convention, 100% denotes no difference. Livers from pregnant animals utilized approximately 50% more radioactive alanine. Concurrent analyses of perfusion medium and liver cells for 'free' alanine indicated that some of the enhanced utilization may have resulted from an increased capacity of the liver of the pregnant rat to transport alanine against a concentration gradient. The augmented labeling of glucose paralleled the absolute increase in glucose production and corroborated that it originated from the alanine in the perfusate. Finally, the

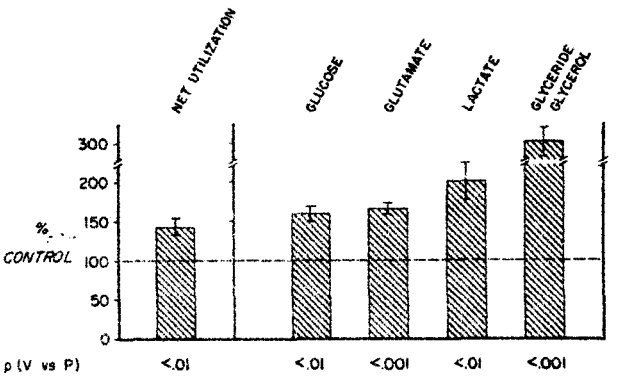

Fig. 4. Effect of pregnancy on ^{14}C-L-alanine disposition during liver perfusion. Net utilization of alanine and its incorporation into the above products were measured during 60 min. perfusion of livers from 24-hour fasted 19-day pregnant and virgin littermate rats. p denotes significant differences per μmole DNA between values in virgin indicated as control (100%) and pregnant rats.

formation of labeled glutamate, lactate, and glycerophosphate (as glyceride-glycerol) was stimulated to a greater degree than total utilization -- a finding which could concord with greater generation of reducing equivalents in the liver of the pregnant rat and disproportionate channeling of carbons along reductive pathways.

Catabolism in the intact animal

The above experiences with 'loading' doses of pyruvate *in vivo* or alanine *in vitro* afford some index of potential gluconeogenic efficiencies. However, they need not equate with the net formation of glucose from endogenous resources in the intact animal. The latter will depend upon the generation of endogenous precursors in the periphery and their rate of delivery to and processing within gluconeogenic sites. To evaluate endogenous events, 24-hour urine collections were secured during free access to food (on days 18–19 of gestation) and two days of fasting (on days 19–21 of gestation) (Freinkel, 1969; Freinkel *et al.*, 1970; Herrera *et al.*, 1969*b*). Paired control urine collections were obtained from age-matched virgin rats. In the fed state, urinary urea was not different in virgin and gravid animals. On the other hand, during the first day of fasting (Fig. 5*a*) nitrogen excretion was significantly greater in the gravid than the non-gravid animal – and the increment was due in large measure to urea, although ammonia excretion was also augmented significantly. Total urinary nitrogen was still significantly greater in pregnant rats on the second day of fasting (Figure 5*b*). However, the increment was now ascribable almost entirely to ammonia.

Do these heightened excretions of nitrogen concord with other parameters of augmented catabolism in the mother? To evaluate this with maximum sensitivity, animals were used as their own controls and urines were monitored for electrolytes during fasting from day 19–21 of gestation and again during fasting from day 10–12 postpartum (Herrera *et al.*, 1969*b*). During each of the two days of fasting, urinary sodium was not significantly different antethan postpartum (Table III). However, the pregnant animals excreted significantly more potassium and phosphorus. The ratios for urinary phosphorus to nitrogen approximated the relationships that obtain in rat muscle (Lilienthal *et al.*, 1950) and hence are compatible with muscle catabolism. On the other hand, the ratios for potassium to nitrogen are greater than in rat muscle (Lilienthal *et al.*, 1950).

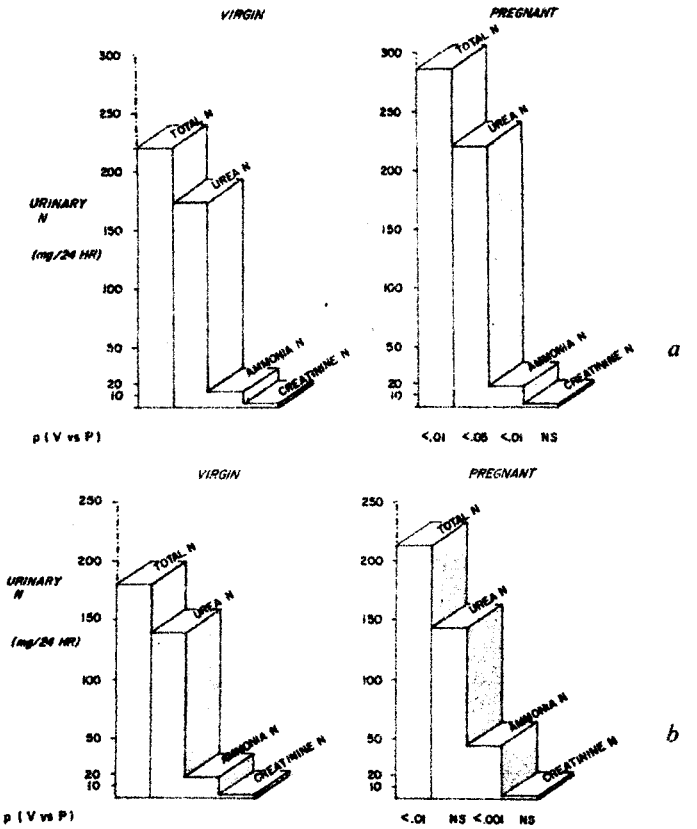

Fig. 5. Effect of pregnancy on nitrogen excretion during fasting in the rat. 24-hour urinary excretion of nitrogenous products was measured in pregnant and age-matched virgin rats. Fig. 5*a* represents day 1 of fast (day 19-20 of gestation) and Fig. 5*b* denotes values during day 2 of fast (day 20-21 of gestation).

TABLE III

Effect of pregnancy on urinary electrolytes during fasting in the rat

	Urinary excretion per 24 hours		P
	AP*	PP**	
Fast day 1			
Sodium (mEq.)	0.59±0.9	0.81±0.17	NS
Potassium (mEq.)	1.53±1.1	1.14±0.14	<.05
Phosphorus (mg)	28.8 ±1.1	18.6 ±2.2	<.01
Fast day 2			
Sodium	0.41±0.07	0.35±0.12	NS
Potassium	1.03±0.16	0.42±0.08	<.05
Phosphorus	21.5 ±0.9	14.8 ±1.4	<.01

* AP = Antepartum (day 19-20 day 1; day 20-21 day 2).
** PP = Postpartum (day 10-11 day 1; day 11-12 day 2).

Possible nitrogen conservation

Do any of our other findings constitute evidence for conservation or reutilization of nitrogen? Definitive answers are not possible at present. However, certain preliminary observations from ongoing studies may be relevant (Metzger *et al.*, 1970; Shambaugh *et al.*, 1971).

According to current concepts, urea arises as a non-utilizable end-product of nitrogen metabolism in liver and meaningful ammoniagenesis is confined to the kidney. Our experiences during perfusion of livers from 24-hour fasted rats suggest that some differences may obtain in pregnancy (Figure 6). Of the nitrogen released as urea or ammonia during 60 minutes' perfusion with 17 mM alanine, fully 70% was present as ammonia in the pregnant animals. In other words, a substantial proportion of the nitrogen evolved during gluconeogenesis in livers from pregnant animals may be released in a form that can be reutilized elsewhere. In association with Dr. George Shambaugh III we have documented the presence of transaminative pathways and mechanisms for the fixation of nitrogen for pyrimidine biosynthesis in the placenta and in fetal tissues (Shambaugh *et al.*, 1971). Thus, if similar hepatic ammoniagenesis obtains *in vivo*, it could provide a mechanism for sustaining growth in the conceptus via the nitrogen derived in the course of maternal gluconeogenesis (Metzger *et al.*, 1970; Shambaugh *et al.*, 1971).

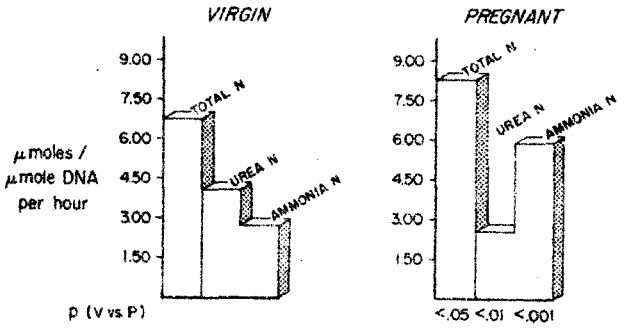

Fig. 6. Effect of pregnancy on production of urea and ammonia during liver perfusion in the rat. Littermate virgin and 19-day pregnant animals were fasted 24 hours prior to perfusion of livers with 17 mM alanine. Total N equals sum of urea and ammonia generated during 60 min. perfusion.

Conclusions and speculations

How can all this be integrated into a cohesive format? As yet, a complete formulation is not possible, but existing information may justify certain speculations (Figure 7). In the preceding section we have focused on the transfer of maternal fuels via the placenta. An additional property of the placenta is its emergence as an endocrine structure. It elaborates hormones such as estrogen and progesterone directly, or secretes principles which sustain ovarian steroid production in species such as the rat. The placenta also synthesizes peptide(s?) which can exhibit luteotropic, mammontropic (Astwood and Greep, 1938) and growth hormone-like properties (Josimovich and MacLaren, 1962). In most species, these secretions parallel the growth of the conceptus (Beck *et al.*, 1965; Kaplan and Grumbach, 1965; Samaan *et al.*, 1966; Spellacy *et al.*, 1966, 1967). More importantly (Spellacy *et al.*, 1967), their elaboration is unremitting and not subject to feedback regulation by the acute excursions in circulating metabolites which differentiate the fed and the fasted states under conditions of normal dietary intake (Samaan *et al.*, 1966; Spellacy *et al.*, 1966).

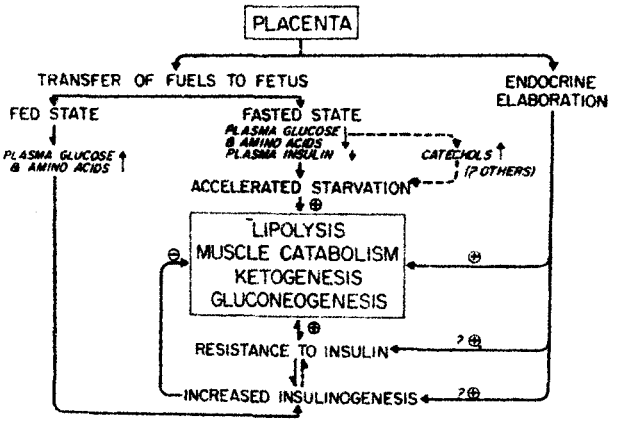

Fig. 7. Postulated pathways through which placental elaboration of hormones and fetal metabolic requirements influence maternal insulin and fuel homeostasis in fed and fasted states.

At present, one cannot assign relative importance to individual hormones nor can one say whether any, or all, directly act upon muscles, liver, tissue responsiveness to insulin, or pancreatic islets. However, in concert, they effect an enhanced turnover of fat in adipose tissue, islet hyperplasia, and they increase the amount of insulin that is required for glucose disposition in the intact animal. In the fed state, the mother elaborates sufficient insulin to maintain glucose homeostasis and to restrain hepatic glucose production and ketogenesis. However, when food is withheld, plasma insulin falls and opposition to the persistent contra-insulin factors is removed. The fall of insulin may even be accelerated by its continued degradation within the placenta (Freinkel and Goodner, 1960). Thus, fat mobilization, muscle catabolism, ketogenesis, gluconeogenesis, and all the adaptations to starvation are 'triggered' more rapidly. Extrauterine mechanisms may be supplemental. For example, we have observed increased elaboration of catecholamines as blood sugar in the fasted pregnant rat approaches the hypoglycemic range (Herrera *et al.*, 1969*a*).

What physiological ends are subserved in this fashion? One could suggest that the potentiality for 'accelerated starvation' (Freinkel, 1965) is protective for mother as well as fetus. With an intermittently eating mother and a continuously feeding fetus, a metabolic setting for rapid transfer to fat would be highly desirable. It would facilitate conservation of maternal glucose and gluconeogenic precursors when exogenous nutrients are withheld and assure their availability for the maternal brain and fetal tissues. If the altered partition between urea and ammonia which we have observed in the perfused liver also obtains *in vivo*, a further conservation of nitrogen could be possible. The increasing placental elaboration of contra-insulin factors in parallel with the growth of the fetus provides just the right temporal juxtaposition to make it all work.

ADDENDUM

Since this manuscript was prepared, Chernick and Novak (*Diabetes*, 1970, *19*, 563) have also reported evidence for enhanced lipolysis in adipose tissue of pregnant rats.

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