not treated with mitomycin C (132,176 count/min as compared to the control of 90 count/min). The allogeneic cells (X) used as stimulating cells verified the ability of A to respond, and the reciprocal test was also shown to be stimulatory (XAm). The allogeneic lymphocytes in the reaction XBm also incorporated [^{3}H]thymidine when incubated with the lymphoblast (Bm).

One possible explanation of the stimulation caused by autologous lymphocytoblasts is that fetal calf antigens derived from the culture medium had become associated with the cultured cells. To test this possibility, lymphoblast culture 2666 was washed twice with medium containing human type A, Rh positive serum and then was cultured in medium in which human serum (same ABO and Rh type as original donor) had been substituted for the fetal calf serum. After 30 days, the lymphoblasts grown in medium containing human serum were assayed for stimulatory activity in the mixed leukocyte culture test (Table 2). These lymphoblasts also stimulated the autologous peripheral lymphocytes (24,214 count/min compared to controls of 603 and 992 count/min), which suggests that antigens derived from fetal calf serum had played no role in the capacity of these cells to stimulate. Furthermore, we were unable to detect bovine antigens on these cells in cytotoxicity tests employing antiserum to fetal calf serum and antiserum to Forssman antigen.

Another possible explanation of the stimulation caused by autologous lymphoblast cells is that the cells elaborate a soluble factor that causes stimulation of the peripheral leukocytes. An attempt was made to determine if the cell-free culture filtrate was stimulatory. No stimulation was found when three dilutions of the culture filtrate were used (2.0, 1.0, and 5.0 ml of filtrate compared to fresh medium; all volumes were adjusted to 2.5 ml with the medium employed in the MLC). This result suggests that a soluble blastogenic factor is not present, but of course such experiments do not rule it out entirely.

The autologous stimulation described here is comparable to the stimulation observed in mixed cultures of lymphocytes from allogeneic individuals possessing different alleles at the HL-A locus. Using methods routinely employed in this laboratory (*6*), we established that there is no difference between the HL-A type of the donor and the HL-A type of his autologous lymphoblast cultures. These observations are in agreement with those of Kourilsky *et al.* (*7*).

The remaining alternative interpretation of this phenomenon is that cells in culture synthesize an antigen not found on the peripheral lymphocyte. Bach *et al.* (*5*), using leukemic cells, suggested that the stimulation found in their laboratory may be due to a leukemic antigen, minor non-HL-A antigens, or a blast cell antigen. It appears from our data that the reaction may be caused by a blast-associated antigen that is not found on normal lymphocytes. Further investigations will be necessary to elucidate the mechanism of this in vitro reaction of autologous cells. Such investigations may be expected to facilitate understanding of the MLC test and its relationships to immunological events.

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10. Since the submission of this report, the work of C. M. Steel and D. A. Hardy [*Lancet* **1970-I**, 1322 (1970)] has come to our attention. They present similar findings using cells cultured from patients with infectious mononucleosis. Our observations first reported here concern the reactions of a cell line derived from a clinically healthy normal donor.

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Starvation in Human Pregnancy: Hypoglycemia, Hypoinsulinemia, and Hyperketonemia

Abstract. *In women fasted during the second trimester of pregnancy, concentrations of glucose and insulin in the plasma fell to a greater extent and ketone acid concentrations in the blood rose more rapidly than in nonpregnant controls. Nitrogen excretion in the urine, particularly ammonia, was increased in the pregnant group. Continuous glucose utilization by the conceptus may exaggerate and accelerate the metabolic consequences of starvation.*

The influence of pregnancy on maternal metabolism has been extensively investigated in human subjects in the postabsorptive and fed states (*1*). However, the metabolic response to periods of fasting extending beyond 12 to 15 hours, although well characterized in nonpregnant humans (*2*) and intensively studied in pregnant rats (*3*), has not been examined in human pregnancy. In view of the obligate requirements of the fetus for glusose (*4*), the changing hormonal milieu characteristic of pregnancy (*5*), and species differences in maternal and fetal metabolism (*4*), observations on nonpregnant individuals or on pregnant laboratory animals may not be applicable to the human gravid state. Furthermore, the potential importance of maternal undernutrition in the genesis of alterations in fetal growth and development (*6*) underscores the need for characterizing the maternal response to caloric deprivation. In our study the metabolic response to an 84-hour fast has been studied in women during the second trimester of pregnancy and compared to that of a nonpregnant control group.

The pregnant subjects were 12

volunteers (16 to 26 years) in good physical health, who were in week 16 to 22 of gestation and were scheduled to undergo therapeutic abortion for psychiatric reasons. The subjects were not asked to participate in the study until after approval for therapeutic abortion had been obtained from a committee of hospital physicians. In obtaining consent, all subjects were informed that therapeutic abortion would be performed regardless of their participation in the study. The control group consisted of six healthy nonpregnant female volunteers (19 to 23 years old). None of the preganant or nonpregnant subjects had a history or evidence of diabetes, thyroid or adrenal disorders, or liver disease. Mean body weight and body surface area were, respectively, 61.7 kg (range 44 to 71 kg) and 1.65 m^2 (range 1.38 to 1.79 m^2) in the pregnant group and 63.9 kg (range 53 to 70 kg) and 1.71 m^2 (range 1.48 to 1.83 m^2) in the nonpregnant group. All subjects were within 14 percent of their ideal body weight (*7*).

The control and preganant subjects were hospitalized at the Clinical Research Center of the Yale–New Haven Hospital. Starvation was initiated after completion of the evening meal at 8 p.m. on the day of admission and was continued for 84 hours (until the morning of day 5 in the hospital). During this time intake was limited to 1200 ml (or more) of water per day. Blood samples were obtained from an antecubital vein between 8 and 9 a.m. on each day of the fast, with the subjects in the recumbent position. Twenty-four-hour collections of urine were initiated at 7 a.m. on the day after admission and were continued throughout the period of starvation.

Glucose was measured in plasma by the glucose oxidase procedure (*8*). Plasma insulin was determined by radioimmunoassay with talc to separate bound and free insulin (*9*). Acetoacetate and β-hydroxybutyrate were determined enzymatically (*10*) on the supernatant fluid of whole blood treated with perchloric acid (*2*). Total nitrogen in the urine was measured by the Kjeldahl technique, urinary urea and ammonia by a colorimetric procedure (*11*), and creatinine by the picric acid method (*12*).

In both groups of subjects, starvation resulted in a fall in the concentrations of glucose and insulin in the plasma and an elevation in the concentration of blood ketone acids (Table 1). However, these changes were more pronounced and occurred with greater rapidity in the pregnant group. Plasma glucose concentrations were consistently lower in the pregnant subjects, falling below 50 mg/100 ml in nine subjects (none, however, had symptoms of hypoglycemia). Similarly, plasma insulin was significantly lower in the pregnant group for the first 60 hours of the fast. Furthermore, whereas the concentration of insulin reached its nadir in the pregnant women within 36 hours, in the nonpregant subjects a continuous decline in insulin was observed over 84 hours before comparable concentrations were reached. In both groups a significant direct linear correlation between plasma insulin and glucose was demonstrable (nonpregnant, $r = .497$, $P < .01$; pregnant, $r = .481$, $P < .01$). The concentrations of acetoacetate and β-hydroxybutyrate were two- to threefold higher in the pregnant subjects for the first 36 to 60 hours of the fast. However, by 84 hours the concentrations of ketone acids were identical in the two groups.

Because maintenance of glucose homeostasis during fasting depends in part on intact mechanisms of protein catabolism and gluconeogenesis (*13*), urinary nitrogen excretion was examined (Table 2). Total nitrogen excretion was comparable in the two groups on days

Table 1. Concentrations of glucose and insulin in the plasma and ketone acid in the blood of nonpregnant and pregnant subjects during an 84-hour fast. Values are mean ± S.E.; not significant, N.S.

Subject	Duration of fast (hours)			
	12	36	60	84
	Glucose (mg/100 ml)			
Nonpregnant	76.2 ± 1.9	69.5 ± 4.2	65.0 ± 3.2	60.7 ± 2.7
Pregnant	66.7 ± 1.4	50.7 ± 1.7	47.6 ± 1.9	46.8 ± 2.5
*P**	< .001	< .001	< .001	< .005
	Insulin (μunit/ml)			
Nonpregnant	11.1 ± 1.1	7.6 ± 0.6	6.8 ± 0.4	4.7 ± 0.4
Pregnant	6.5 ± 0.6	4.1 ± 0.4	4.5 ± 0.7	4.9 ± 0.5
*P**	< .005	< .001	< .02	N.S.
	Acetoacetate (mmole/liter)			
Nonpregnant	0.06 ± 0.01	0.29 ± 0.07	0.71 ± 0.03	0.82 ± 0.03
Pregnant	0.15 ± 0.03	0.65 ± 0.06	0.71 ± 0.05	0.91 ± 0.04
*P**	< .01	< .005	N.S.	N.S.
	β-Hydroxybutyrate (mmole/liter)			
Nonpregnant	0.10 ± 0.05	1.09 ± 0.38	2.44 ± 0.20	4.03 ± 0.23
Pregnant	0.37 ± 0.04	2.04 ± 0.19	2.99 ± 0.14	4.21 ± 0.19
*P**	< .005	< .05	< .05	N.S.

* Significance of differences between mean values for nonpregnant and pregnant subjects.

Table 2. Influence of pregnancy on urinary nitrogen excretion during starvation. The results are expressed as the number of grams excreted per unit of body area (m^2) per 24 hours (mean ± S.E.).

Subject	Day of fast		
	Day 1	Day 2	Day 3
	Total nitrogen		
Nonpregnant	4.5 ± 0.5	4.8 ± 0.7	5.4 ± 0.5
Pregnant	4.8 ± 0.2	5.6 ± 0.3	6.8 ± 0.3
*P**	N.S.	N.S.	< .03
	Urea nitrogen		
Nonpregnant	3.7 ± 0.5	4.1 ± 0.7	4.5 ± 0.5
Pregnant	3.9 ± 0.2	4.3 ± 0.2	5.4 ± 0.2
*P**	N.S.	N.S.	N.S.
	Ammonia nitrogen		
Nonpregnant	0.41 ± 0.14	0.34 ± 0.06	0.53 ± 0.09
Pregnant	0.35 ± 0.03	0.60 ± 0.08	1.02 ± 0.10
*P**	N.S.	< .02	< .005
	Creatinine nitrogen		
Nonpregnant	0.26 ± 0.02	0.26 ± 0.01	0.26 ± 0.02
Pregnant	0.25 ± 0.01	0.28 ± 0.02	0.28 ± 0.02
*P**	N.S.	N.S.	N.S.

* Significance of differences between mean values for nonpregnant and pregnant subjects.

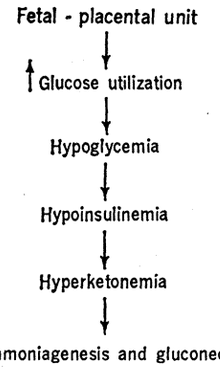

Fig. 1. Postulated sequence whereby glucose utilization by the fetal-placental unit alters metabolic response to starvation in midpregnancy in humans. The hyperketonemia resulting from lack of insulin may be mediated through increased lipid mobilization.

1 and 2 of the fast and was significantly elevated in the pregnant group on day 3. This increase was primarily due to an elevation in urinary ammonia excretion which rose to concentrations twice those observed in nonpregnant controls. In view of the comparable concentrations of urinary urea and creatinine in the two groups it is unlikely that the increase in glomerular filtration rate observed in pregnancy was responsible for the augmented excretion of total nitrogen and ammonia.

Total weight loss during the fast was 3.1 ± 0.4 kg (mean $\pm$ S.E.) in the nonpregnant group and 3.2 ± 0.2 kg in the pregnant subjects.

The current data demonstrate that the response of circulating glucose, insulin, and ketone acids to total starvation is exaggerated and accelerated in human subjects during the second trimester of pregnancy. The striking decline in plasma glucose to hypoglycemic concentrations could be attributed to overutilization of glucose or underproduction of glucose from endogenous precursors, or both. The normal to increased rates of protein dissolution suggest that maternal gluconeogenic mechanisms are intact. Moreover, in view of the interdependence of renal ammoniagenesis and gluconeogenesis (*14*), the elevation in urinary ammonia excretion is consistent with an augmentation in renal gluconeogenesis. Thus fasting hypoglycemia appears to be a consequence of continuous glucose consumption by the conceptus. That the marked reduction in plasma glucose is in turn responsible for the hypoinsulinism is suggested by the direct correlation between plasma glucose and insulin concentrations and the fact that insulin and glucose concentrations reach a simultaneous plateau at 36 hours.

With respect to the hyperketonemia of pregnancy, the importance of placental diabetogenic, contrainsulin factors has been postulated in both man (*5*) and laboratory animals (*15*). That such is not the case in midpregnancy in humans is suggested by the absolute hypoinsulinism in the pregnant group. The heightened ketonemia of pregnancy is thus more readily explained on the basis of the reduction in plasma insulin concentrations. Supporting this conclusion is the demonstration that acetoacetate and β-hydroxybutyrate concentrations were higher in the pregnant group so long as plasma insulin concentrations were significantly below those of nonpregnant controls (Table 1). However, when plasma insulin declined in the nongravid group after 84 hours of starvation to the concentration observed in pregnant women, ketone acid concentration in blood rose to virtually identical levels in the two groups. These effects of the lack of insulin on ketosis may be mediated through increased mobilization of free fatty acids. Finally, because ketonuria in starvation is dependent on the concentration of blood ketones and in turn influences the rate of ammonia excretion (*14*), hyperketonemia is the likely explanation for the heightened excretion of ammonia in the gravid state.

The sequence of events postulated to account for the altered fuel-hormone response to starvation in human subjects in the second trimester of pregnancy is shown schematically in Fig. 1. A similar concept has previously been advanced on the basis of observations in fasted gravid rats (*3*) and in postabsorptive women in the third trimester of pregnancy (*1*). However, the demonstration of hyperinsulinemia in those situations suggests a role for contrainsulin factors which appears to be of less importance in human midpregnancy.

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Centrifugal Effects in the Avian Retina

Abstract. *Electrical stimulation of the centrifugal fibers to the avian retina can disturb the balance between the excitatory and inhibitory system within the receptive fields of individual retinal ganglion cells. Although the mechanisms may vary from one unit to another, the effect is always to make them fire more readily and to a wider range of visual inputs.*

The bird is the only vertebrate for which there is conclusive evidence of an efferent retinal projection (*1*). The cell bodies of the centrifugal neurons are collected in the isthmo-optic nucleus, and their axons run forward together in the isthmo-optic tract, joining the optic nerve at the chiasma. Their endings terminate chiefly on the amacrine cells, which in the bird retina are probably genuine interneurons linking bipolars and ganglion cells, and are therefore well situated to modulate the flow of information through the retina. These isthmo-optic neurons compose the efferent limb of a local feedback