Human Maternal and Fetal Serum Insulin and Growth Hormone (HGH) Response to Glucose and Leucine

S. Grasso, M. D., G. Palumbo, M. D., A. Messina, M. D., C. Mazzarino, Sc. D., and G. Reitano, M. D., Catania, Italy

With the technical assistance of L. Lo Nigro

SUMMARY

Maternal and fetal serum insulin and HGH responses to glucose, leucine, and glucose plus leucine were examined by infusions to pregnant women at term immediately before cesarean section. Leucine (15 gm.) with glucose (50 gm.) administered for 30 minutes to the mothers stimulates markedly maternal and fetal insulin secretion while infusion of glucose (50 gm.) causes a lower insulin response. When infusing glucose alone we noted that the duration rather than the degree of hyperglycemia determined the fetal insulin response. In fact, when glucose is given to the mother for 60 minutes the fetal insulin response is higher than when the same dose is infused for 30 minutes. Maternal infusion of leucine (15 gm.) for 30 minutes elicits only a very slight increase of insulin secretion in the mother and no change in the fetus. None of the infusions causes any alteration whatsoever in either maternal or fetal HGH secretion. DIABETES 25:545-49, July, 1976.

We have shown that a mixture of essential amino acids given over a period of 30 minutes stimulates insulin secretion in the premature infant.[1] When arginine is given alone to premature[2] and term infants[3] it causes a modest rise in insulin levels, but when this amino acid is infused with glucose these two stimuli have a synergistic effect and produce a large serum insulin response.[2] A similar synergism is observed between glucose and a mixture of essential amino acids.[4] These amino acids, with or without glucose, stimulate HGH secretion in the premature infant.[2,5] In contrast, most studies show that the neonatal pancreas responds poorly to an increase of blood glucose concentration.[5]

To further extend these studies, both maternal and fetal insulin and HGH response were determined by infusing glucose or leucine, with or without glucose, into normal pregnant women at term undergoing cesarean section. Leucine was chosen as it is an insulinogenic amino acid that also causes a small rise in serum HGH in the adult.[6,7]

While carrying out this study we kept in mind that neither maternal nor fetal HGH[8] and insulin[9] cross the placenta.

METHODS

Sixty-three pregnant women who underwent cesarean section consented to participate in this study. Such sections were done for the following reasons: (1) previous sections—42 patients, (2) disproportion —five patients, (3) transverse lie—10 patients, (4) advanced age of the mother—six patients. None had a history of diabetes or were obese. They were chosen because the fetuses didn't undergo the stress of labor.

All studies were performed in the morning after 18-20 hours' fast. A 20-gauge needle was placed in the right antecubital vein, and one of the following substances was administered: (1) glucose, 50 gm.; (2) L-leucine, 15 gm.; (3) glucose, 50 gm. plus L-leucine 15 gm.; (4) saline. Glucose was administered as a 10 per cent solution. When glucose was mixed with L-leucine, 100 ml. of the 50 per cent glucose was added to 750 ml. of the amino acid solution. The infusion lasted 30 minutes and was synchronized so

From the Department of Morbid Anatomy, Obstetrics and Gynecology (Osp. S. Bambino), General Pathology, and Pediatrics, University of Catania, Italy.

Address reprint requests to Dr. G. Reitano, Clinica Pediatrica, Viale A. Doria, Catania, Italy.

Accepted for publication February 26, 1976.

that birth occurred 10 minutes after it ended. In nine patients the administration of glucose lasted 60 minutes, and in 10 others saline was infused for the same duration as a control. None of the mothers or the newborns suffered any ill-effect from the infusion of these substances. Maternal venous blood samples were taken at 0 and 30 minutes, and at 60 minutes from those infused for that length of time. Maternal venous and fetal umbilical mixed blood samples were obtained simultaneously at delivery.

All mothers received the premedication of atropine and promethazine. Anesthesia was induced by thiopenthal sodium and maintained by 50 per cent nitrous oxide and 50 per cent oxygen and succinylcholine.

Blood glucose was determined by a glucose oxidase method. Serum insulin and HGH were determined in triplicate by radioimmunoassay methods.[10,11]

RESULTS

Effects of maternal infusion of saline or leucine (15 gm.) for 30 minutes on mother and fetus (figure 1). After infusion of either saline (n=12) or leucine (n=4) to the mothers no significant alteration in maternal or fetal glucose levels was noted. While leucine brought about a small rise in the maternal serum insulin at 30 minutes, saline had no effect at all. At delivery, umbilical blood glucose, serum insulin, and HGH were 61 ± 3 mg. per 100 ml. (mean ± S.E.M.), 9 ± 2 μU. per ml., and 15 ± 2 ng. per ml. in the saline control group; 70 ± 2 mg. per 100 ml., 15 ± 2 μU. per ml., and 24 ± 5 ng. per ml. in the leucine-infused group. The mean body weight of the infants born to the mothers infused with saline was 3,445 ± 150 gm.; with leucine 3,405 ± 25 gm. In the mothers infused with saline, blood glucose was 79 ± 3 mg. per 100 ml. at 0 minutes and 84 ± 3 mg. per 100 ml. at 30 minutes, while serum insulin and HGH were respectively 8 ± 1 μU. per ml. and 6 ± 0.3 ng. per ml. at 0 minutes and 7 ± 1 μU. per ml. and 6 ± 0.3 per ml. at 30 minutes. In the mothers infused with leucine, blood glucose was 82 ± 5 mg. per 100 ml. at 0 minutes and 88 ± 5 mg. per 100 ml. at 30 minutes, while serum insulin and HGH were respectively 9 ± 2 μU. per ml. and 7 ± 2 ng. per ml. at 0 minutes and 18 ± 2 μU. per ml. and 7 ± 2 ng. per ml. at 30 minutes.

Effects of maternal infusion of glucose (50 gm.) for 30 minutes on mother and fetus (figure 1). The fetal and maternal pancreas responded to glucose stimulus with an increase of insulin output. At delivery after this administration of glucose to the mothers (n=11) the serum insulin was 26 ± 6 μU. per ml., while the blood glucose was 236 ± 6 mg. per 100 ml. in the umbilical blood. This insulin level was significantly higher than that in the control group (p less than 0.01). The fetal serum HGH was 20 ± 3 ng. per ml. and did not differ significantly from that of the control group. The mean body weight of the infants was 3,385 ± 123 gm. At 30 minutes the maternal serum insulin level during the infusion rose from a control level of 9 ± 2 to 70 ± 14 μU. per ml., while the blood glucose rose from 84 ± 7 to 333 ± 5 mg. per 100 ml. At delivery maternal serum insulin was 59 ± 10 μU. per ml. and the blood glucose was 293 ± 9 mg. per 100 ml. The serum HGH was 6 ± 0.5 ng. per ml. at 0 minutes and 6 ± 0.5 and 7 ± 0.7 ng. per ml. at 30 minutes and at delivery, respectively.

Effects of maternal infusion of glucose (50 gm.) with leucine (15 gm.) for 30 minutes on mother and fetus (figure 1). A potent stimulus of fetal and maternal pancreatic

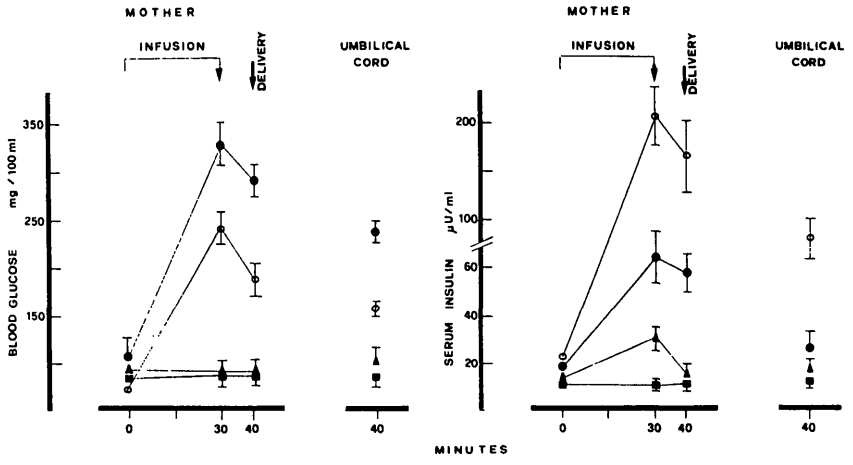

FIGURE 1

Comparison of maternal and fetal blood glucose and serum insulin levels (± S.E.M.) in pregnant women at term infused for 30 minutes with saline (■ ---■), leucine (▲---▲), glucose (●---●), or glucose in combination with leucine (○---○).

insulin secretion followed the maternal infusion of glucose in combination with leucine (n = 17). At delivery our results showed that the serum insulin was 88 ± 15 μU. per ml. and the blood glucose was 157 ± 5 mg. per 100 ml. in the umbilical blood. This insulin value was significantly higher than that in fetuses of the mothers receiving glucose alone (p less than 0.01). The serum HGH in the umbilical blood was 22 ± 4 ng. per ml. and did not differ from that of the control group. The mean body weight of the infants was 3,495 ± 92 gm. During the infusion the maternal serum insulin rose from a control level of 16 ± 4 to 202 ± 25 μU. per ml. while the blood glucose increased from 74 ± 3 to 242 ± 8 mg. per 100 ml. at 30 minutes. At delivery maternal serum insulin was 165 ± 30 μU. per ml. and blood glucose was 187 ± 7 mg. per 100 ml. The serum HGH was 8 ± 0.6 ng. per ml. at 0 minutes and 8 ± 0.6 and 9 ± 0.8 ng. per ml. at 60 minutes and at delivery, respectively.

Effects of maternal infusion of glucose (50 gm.) or saline for 60 minutes on mother and fetus (figure 2). On infusion of 50 gm. of glucose for 60 minutes, a high fetal insulin response was seen. In fact, in the umbilical blood the serum insulin was 46 ± 8 μU. per ml. while the blood glucose was 187 ± 13 mg. per 100 ml. Although this mean serum insulin level was lower than that in the fetuses of the mothers receiving glucose plus leucine for 30 minutes, there was no statistical difference. However, this insulin value was significantly higher than seen when the same amount of glucose was infused for 30 minutes (p less than 0.05) while the blood glucose was lower (p less than 0.01). The serum HGH in the umbilical blood was 23 ± 4 ng. per ml. and did not differ from that in the control group. The mean body weight of the infants was 3,340 ± 113 gm. During the infusion the maternal serum insulin level rose from a control level of 17 ± 3 to 82 ± 13 μU. per ml. while the blood glucose increased from 77 ± 3 to 237 ± 9 mg. per 100 ml. at 60 minutes. At delivery maternal serum insulin was 55 ± 11 μU. per ml. and blood glucose was 207 ± 11 mg. per 100 ml. The serum HGH was 6 ± 0.7 per ml. at 0 minutes and 6 ± 0.7 and 8 ± 1 ng. per ml. at 60 minutes and at delivery, respectively.

The maternal infusion of saline (n = 10) did not cause any significant alteration in blood glucose, serum insulin, and HGH in either mother and fetus. At delivery the umbilical blood glucose, serum insulin, and HGH were 64 ± 3 mg. per ml., 12 ± 2 μU. per ml., and 26 ± 4 ng. per ml., respectively. The mean body weight of the infants was 3,290 ± 127 gm. The maternal blood glucose was 72 ± 3 mg. per

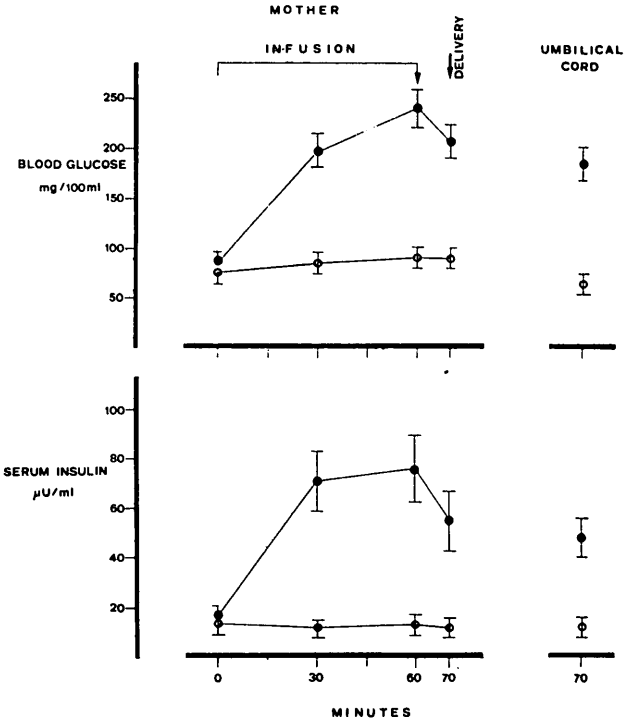

FIG. 2. Comparison of maternal and fetal blood glucose and serum insulin levels (± S.E.M.) in pregnant women at term infused for 60 minutes with saline (0---0) or glucose (●---●).

100 ml. at 0 minutes and 82 ± 4 mg. per 100 ml. at 60 minutes. Serum insulin and HGH were, respectively, 16 ± 3 μU. per ml. and 7 ± 0.5 ng. per ml. at 0 minutes and 13 ± 2 μU. per ml. and 7 ± 0.4 ng. per ml. at 60 minutes.

DISCUSSION

The present data provide evidence that leucine with glucose administered to pregnant women at term, immediately before cesarean section, is a potent stimulus of maternal and fetal insulin secretion. After administering glucose the insulin response is lower in both. Our dose of leucine alone causes only a very slight increase of insulin secretion in the mother and no change in the fetus. None of the infusions cause any alteration whatsoever in either maternal or fetal HGH levels.

The question of whether there are any changes in the pattern of insulin and HGH release during pregnancy has been investigated by several authors. It has been reported that the plasma insulin response to an intravenous glucose load augments during the last 18 weeks of pregnancy[12,13] The infusion of arginine

causes an attenuated insulin response in early pregnancy that improves as term approaches,[14] while the ingestion of leucine elicits a rise in plasma insulin concentration similar to that found in nonpregnant women.[15] As in normal subjects,[16] the simultaneous administration of glucose and leucine to our patients causes an increase in serum insulin greater than does the separate infusion of these substances. The higher levels of serum insulin observed during the administration of the glucose-leucine mixture are not the result of a greater increase in blood glucose. In fact, during the infusion of the glucose-amino acid mixture, the blood glucose levels are lower than when glucose is infused alone.

The absence of HGH response to the leucine with glucose infusion in our pregnant women was foreseen. In normal subjects, the release of this hormone induced by the intravenous infusion of essential amino acids is suppressed by the administration of glucose.[17] In our patients, when leucine is given alone, the dose is probably too small to cause any alteration in HGH secretion, and, moreover, no increase was noted because our studies lasted only 40 minutes. It is known that the increase in HGH release in normal subjects is seen after 60 minutes.[7] Futhermore, pregnant women show a sluggish HGH secretory response to most stimuli in the last trimester of pregnancy.[14,18]

Previous studies have demonstrated that early in pregnancy (13 to 26 weeks' gestation) fetal insulin response to maternal hyperglycemia is negligible.[9,19] By term the fetus in utero is capable of responding to maternal hyperglycemia with an elevation of serum insulin, though the responses are attenuated compared with those of adults.[20] In the present study we noted that the duration, rather than the degree of hyperglycemia, determined the fetal insulin response. In fact, when glucose is given to the mother for 60 minutes, the fetal insulin response is higher, despite the lower blood glucose, than when the same dose is infused for 30 minutes. After giving a rapid glucose infusion to normal women in labor, Cordero et al.[21] observed an increase in fetal blood glucose that declined slowly, and a delayed increase in serum insulin. The highest insulin values are reached approximately 60 minutes after the glucose peak. This has been reported by other authors.[22,23] Apparently the normal fetus adapts to a rapid increment in its blood glucose level with a gradual increase in insulin secretion.

The administration of glucose with leucine to our pregnant women provokes an even greater fetal insulin response. However, it must be underlined that, even if the mean fetal serum insulin level following a 60-minute maternal infusion of glucose was lower than that in fetuses of the mothers receiving glucose plus leucine, it was not statistically different.

The data concerning the role of amino acids on insulin and HGH secretion in the fetus are conflicting. It has been reported that isolated human fetal pancreatic tissue from 14 to 20 weeks of gestation releases insulin when incubated with leucine or arginine but not with glucose.[24] Mintz et al.[25] on infusing monkey fetuses observed that a mixture of essential amino acids causes an increase of insulin secretion, while arginine and glucose do not. The infusion of arginine to pregnant women early in gestation or at term during active labor does not cause any significant serum insulin rise in their fetuses.[26] Contrastingly, studies have shown that the infusion of this amino acid to pregnant women at term prior to the onset of labor provokes a small but significant increase in fetal plasma insulin.[27] In the neonate, leucine appears an ineffective stimulant of insulin secretion.[28] Comparisons between our data and those of the literature are difficult to draw. This could be due to the different technics of studying insulin release in the fetus and/or the selection of the amino acid used. In fact, it is known that the mechanism by which leucine induces insulin release from the pancreatic islets differs from that of other essential amino acids.[29] In the premature infant it has been shown that glucose and amino acids are synergistic in their insulin-stimulatory action.[4] Hopkins et al.[30,31] on studying placental transport of free amino acids in animals have demonstrated that the branched-chain neutral amino acids, such as leucine, belonging to the "L-preferring" group of Christensen are the most readily transported across the placental membranes.

HGH is present in the human fetus at an early stage of gestation, and high levels of this hormone in the fetus and newborn have been reported.[32] At term following maternal glucose infusion, a fall in fetal plasma HGH has been shown by some authors,[28,33] while the administration of arginine provokes a small rise in the secretion of this hormone in some fetuses.[28] The plasma HGH concentration of fetal monkeys does not change in response to glucose or arginine.[34] In our studies no appreciable alteration has been noted after any of our infusions. It should be pointed out that HGH is extremely variable in the fetus and that in our control groups it ranged from 5 to 51 ng. per ml. Conclusions must be guarded owing to this liability.

As our studies were carried out on pregnant women after a long fast, we do not know what effect this has on fetal insulin and HGH secretion. However, it could be suggested that some amino acids, such as leucine, with glucose may contribute to the regulation

of fetal insulin secretion. Recently it has been demonstrated that the intravenous administration of alanine to normal women during labor causes fetal glucagon secretion.[35]

ACKNOWLEDGMENT

We are indebted to Mrs. Moira Macpherson Grasso for her help in editing this paper.

REFERENCES

[1]Grasso, S., Saporito, N., Messina, A., and Reitano, G.: Serum-insulin response to glucose and aminoacids in the premature infant. Lancet *2*:755, 1968.

[2]Reitano, G., Grasso, S., Distefano, G., and Messina, A.: The serum insulin and growth hormone response to arginine and arginine with glucose in the premature infant. J. Clin. Endocrinol. Metab. *33*:924, 1971.

[3]King, K. C., Adam, P. A. J., Yamaguchi, K., and Schwartz, R.: Insulin response to arginine in normal newborn infants and infants of diabetic mothers. Diabetes *23*:816, 1974.

[4]Grasso, S., Messina, A., Distefano, G., Vigo, R., and Reitano, G.: Insulin secretion in the premature infant. Response to glucose and amino acids. Diabetes *22*:349, 1973.

[5]Grasso, S., Messina, A., Distefano, G., Vigo, R., and Reitano, G.: Regulation of insulin and HGH secretion in the premature infant. *In* Diabetes. Proc. 8th Congr. Inter. Diab. Fed. Brussels 1974 (Malaisse, W. J., and Pirart, J., Eds.) ICS 312 pg. 489. Amsterdam, Excerpta Medica.

[6]Floyd, J. C., Jr., Fajans, S. S., Conn, J. W., Knopf, R. F., and Rull, J.: Stimulation of insulin secretion by amino acids. J. Clin. Invest. *45*:1487, 1966.

[7]Knopf, R. F., Conn., J. W., Fajans, S. S., Floyd, J. C., Jr., Guntsche, E. M., and Rull, J. A.: Plasma growth hormone response to intravenous administration of amino acids. J. Clin. Endocrinol. Metab. *25*:1140, 1965.

[8]Laron, Z., Pertzelan, A., Mannheimer, S., Goldman, J., and Guttmann, S.: Lack of placental transfer of human growth hormone. Acta Endocrinol. *53*:687, 1966.

[9]Adam, P. A. J., Teramo, K., Raiha, N., Gitlin, D., and Schwartz, R.: Human fetal insulin metabolism early in gestation. Response to acute elevation of the fetal glucose concentration and placental transfer of human insulin-I-131. Diabetes *18*:409, 1969.

[10]Hales, C. N., and Randle, P. J.: Immunoassay of insulin with insulin-antibody precipitate. Biochem. J. *88*:137, 1963.

[11]Schalch, D. S., and Parker, M. L.: A sensitive double antibody immunoassay for human growth hormone in plasma. Nature *203*:1141, 1964.

[12]Spellacy, W. N., and Goetz, F. C.: Plasma insulin in normal late pregnancy. N. Engl. J. Med. *268*:988, 1963.

[13]Kalkhoff, R. K., Richardson, B. L. and Beck, P.: Relative effects of pregnancy, human placental lactogen and prednisolone on carbohydrate tolerance in normal and subclinical diabetic subjects. Diabetes *18*:153, 1969.

[14]Tyson, J. E., Rabinowitz, D., Merimee, T. J., and Friesen, H.: Response of plasma insulin and human growth hormone to arginine in pregnant and postpartum females. Am. J. Obstet. Gynec. *103*:313, 1969.

[15]Bleicher, S. J., O'Sullivan, J. B., and Freinkel, N.: Carbohydrate metabolism in pregnancy. V. The interrelations of glucose, insulin, and free fatty acids in late pregnancy and post partum. N. Engl. J. Med. *271*:866, 1964.

[16]Floyd, J. C., Jr., Fajans, S. S., Pek, S., Thiffault, C. A., Knopf, R. F., and Conn, J. W.: Synergistic effect of essential amino acids and glucose upon insulin secretion in man. Diabetes *19*:109, 1970.

[17]Boden, G., Soeldner, J. S., Steinke, J., and Thorn, G. W.: Serum human growth hormone to i.v. glucose: diagnosis of acromegaly in females and males. Metabolism *17*:1, 1968.

[18]Yen, S. S. C., Vela, P., and Tsai, C. C.: Impairment of growth hormone secretion in response to hypoglycemia during early and late pregnancy. J. Clin. Endocrinol. Metab. *31*:29, 1970.

[19]Thorell, J. I.: Plasma insulin levels in normal human foetuses. Acta. Endocrinol. *63*:134, 1970.

[20]Obenshain, S. S., Adam, P. A. J., King, K. C., Teramo, K., Raivio, K. O., Raiha, N., and Schwartz, R.: Human fetal insulin response to sustained maternal hyperglycemia. N. Engl. J. Med. *283*:566, 1970.

[21]Cordero, L., Jr., Grunt, J. A., and Anderson, G. G.: Hypertonic glucose infusion during labor. Maternal-fetal serum insulin relationships. Am. J. Obstet. Gynec. *107*:560, 1970.

[22]Milner, R. D. G., and Hales, C. N.: Effect of intravenous glucose on concentration of insulin in maternal and umbilical-cord plasma. Br. Med. J. *1*:284, 1965.

[23]Oakley, N. W., Beard, R. W., and Turner, R. C.: Effect of sustained maternal hyperglycemia on the fetus in normal and diabetic pregnancies. Br. Med. J. *1*:466, 1972.

[24]Milner, R. D. G., Ashworth, M. A., and Barson, A. J.: Insulin release from human foetal pancreas in response to glucose, leucine and arginine. J. Endocrinol. *52*:497, 1972.

[25]Mintz, D. H., Chez, R. A., and Hutchinson, D. L.: Subhuman primate pregnancy complicated by streptozotocin-induced diabetes mellitus. J. Clin. Invest. *51*:837, 1972.

[26]King, K. C., Butt, J., Raivio, K., Raiha, N., Roux, J., Teramo, K., Yamaguchi, K., and Schwartz, R.: Human maternal and fetal insulin reponse to arginine. N. Engl. J. Med. *285*:607, 1971.

[27]Turner, R. C., Oakley, N. W., and Beard, R. W.: Human fetal plasma growth hormone prior to the onset of labour. Effects of stress, glucose, arginine, and maternal diabetes. Biol. Neonate *22*:169, 1973.

[28]Stimmler, L., Brazie, J. V., and O'Brien, D.: Plasma-insulin levels in the newborn infants of normal and diabetic mothers. Lancet *1*:137, 1964.

[29]Fajans, S. S., Floyd, J. C., Jr., Knopf, R. F., Guntsche, E. M., Rull, J. A., Thiffault, C. A., and Conn, J. W.: A difference in mechanism by which leucine and other amino acids induce insulin release. J. Clin. Endocrinol. *27*:1600, 1967.

[30]Hopkins, L., McFadyen, I. R., and Young, M.: The free amino acid concentrations in maternal and foetal plasmas in the pregnant ewe. J. Physiol. *215*:9, 1971.

[31]Hopkins, L., McFadyen, I. R., and Young, M.: Placental transfer and foetal uptake of free amino acids in the pregnant ewe. J. Physiol. *215*:11, 1971.

[32]Kaplan, S. L., Grumbach, M. M., and Shepard, T. H.: The ontogenesis of human fetal hormones. I. Growth hormone and insulin. J. Clin. Invest. *51*:3080, 1972.

[33]Aubert, M. L., Sistek, J., Chabot, V., and Bossart, H.: L'hormone de croissance hypophysaire foetale pendant l'accouchement. Schweiz. Med. Wochenschr. *101*:1102, 1971.

[34]Mintz, D. H., Chez, R. A., and Horger, III, E. O.: Fetal insulin and growth hormone metabolism in the subhuman primate. J. Clin. Invest. *48*:176, 1969.

[35]Wise, J. K., Lyall, S. S., Hendler, R., and Felig, P.: Evidence of stimulation of glucagon secretion by alanine in the human fetus at term. J. Clin. Endocrinol. *37*:345, 1973.