exhibit composite structures that resulted from coalescence and fusion of smaller agglomerates. The degree of fusion varies over the full range from weak adherence to complete assimilation (*6*).

This is the Godel phenomenon. The tendency of ash to sinter above about 1050°C, which has constrained fluidized bed gasification, has been put to constructive use in the Ignifluid boiler. Separation of ash and coke is nearly complete. Some small fragments and residues of coke are trapped or become embedded in the glass matrix of the ash agglomerates, accounting for a carbon content of about 5 percent. Carbon losses are minor elsewhere. For a coal feed that contains 20 percent mineral matter, clinkers rejected at 5 percent carbon represent carbon utilization of 99 percent.

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References and Notes

1. The following articles provide further description of the Ignifluid boiler: A. Godel and P. Cosar, *AIChE Symp. Ser.* **67**, 210 (1967); A. M. Squires, *Science* **169**, 821 (1970); *Sci. Am.* **227**, 26 (October 1972); J. Yerushalmi and A. M. Squires, in *Conference Proceedings, Power Generations—Clean Fuels Today*, Monterey, Calif., 8 to 10 April 1974 (EPRI-SR-1, Special Report Clean Fuels Conference) (Electric Power Research Institute, Monterey, Calif., 1974), p. 45.
2. A. M. Squires, *Science* **184**, 340 (1974).
3. J. W. R. Rayner, *J. Inst. Fuel* **25**, 50 (1952).
4. P. Nicholls and W. T. Reid, *Trans. Am. Soc. Mech. Eng.* **62**, 141 (1940).
5. The carryover material is collected by mechanical means and recycled to the gasification bed.
6. The characteristics of the ash agglomerates vary widely depending on the type of feed coal and the design, size, and operating conditions of the particular Ignifluid installation. A detailed discussion of this subject will be presented in a monograph on the Ignifluid boiler and the Godel phenomenon (J. Yerushalmi and A. M. Squires, in preparation).
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Maternal Malnutrition and Placental Transfer of α-Aminoisobutyric Acid in the Rat

Abstract. *In the pregnant rat, dietary protein restriction reduces the transfer of ^{14}C-labeled α-aminoisobutyric acid from the maternal blood into the fetus. One of the causes of this phenomenon is a reduced capacity of the placenta to release into the fetus α-aminoisobutyric acid taken up from the maternal blood.*

Maternal restriction of protein and calories is known to cause retarded growth of the fetus in several species of eutherian mammals including man. The mechanisms mediating this effect are still poorly understood. Hammond in 1944 put forward the theory that the division of nutrients between the mother and the fetus is regulated by the metabolic rate of the fetal and maternal tissues (*1*). According to this theory, if there is only a limited supply of nutrients the fetus would be able to compete successfully with the mother because of the higher metabolic rate of its tissues. This implies that the fetus would be affected only after maternal reserves have been depleted. However, recent data from human populations demonstrate that even a moderate restriction of nutrients during pregnancy may cause some degree of fetal growth retardation (*2*).

Thus, in apparent discrepancy with Hammond's hypothesis, when maternal malnutrition is present, the fetus does not seem able to compete successfully with the mother. In order to explain this phenomenon, it seems necessary to consider other factors, besides the metabolic rate of the tissues, in the division of nutrients. One of these factors could be placental function. If maternal malnutrition affects the capacity of the placenta to transfer nutrients, the possibility of fetal competition would be neutralized and fetal growth retardation would result. There is evidence from observations on human populations and experiments in rats that maternal malnutrition interferes with cellular growth of the placenta and alters RNA metabolism (*3*). Moreover, morphometric studies of placenta from women suffering mild degrees of malnutrition demonstrate that malnutrition reduces the mass of peripheral villi (*4*). This

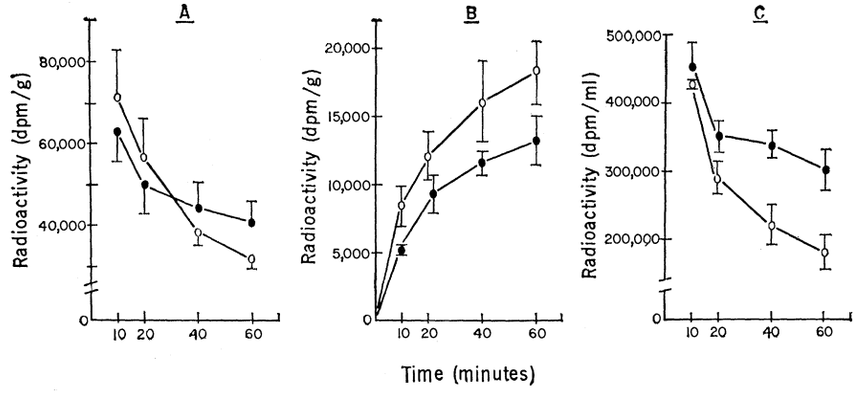

Fig. 1. (A) Concentration of ^{14}C-labeled AIB [disintegrations per minute (dpm) per gram of tissue] in placental tissue of normal (○) and malnourished (●) rats at different times after maternal intravenous administration of 1 μc per 100 g of body weight of α-amino[1-^{14}C]isobutyric acid (12.2 μc/mmole). Each point represents the mean ± the standard error of five placentas in the normal group and four placentas in the malnourished group. A total of five and four animals were used for the normal and malnourished, respectively, in consecutive experiments. Placentas were removed, and a 10 percent homogenate was prepared in distilled water. Radioactivity was measured in a 0.5-ml sample of the homogenate after digestion at 40°C with 2 ml of NCS solubilizer (Amersham/Searle). The digested sample was then neutralized with 0.1 ml of glacial acetic acid, and 15 ml of a scintillation fluid containing 0.01 percent POPOP and 0.4 percent PPO in toluene were added to the vials. (B) Concentration of [^{14}C]AIB (disintegrations per minute per gram of tissue) in fetuses of normal (○) and malnourished (●) rats. The number of cases in each point and the preparation of samples were similar to placentas. (C) Concentration of [^{14}C]AIB (dpm/ml) in the plasma of normal (○) and malnourished rats (●). Radioactivity was measured in 10 μl of plasma digested at room temperature with 0.2 ml of NCS solubilizer. As in previous samples 15 ml of the same scintillation fluid were used.

finding is compatible with a reduced overall capacity of the placenta to take up nutrients and, consequently, with a reduced transfer of nutrients to the fetus. We report here that in malnourished rats there is a reduced transfer of α-aminoisobutyric acid (AIB) from the maternal circulation into the fetus and that one of the causes of this reduction is an altered placental transfer of this amino acid.

Pregnant rats (Sprague-Dawley) were fed either an experimental, 6 percent, or a control, 27 percent, casein diet (*5*) from day 6 of pregnancy. Transfer of AIB from the maternal blood into the fetus was studied by a method similar to the one described by Wapnir and Dierks-Ventling (*6*) with the use of ^{14}C-labeled AIB. At day 20 of pregnancy, under urethane anesthesia (200 mg per 100 g of body weight), each rat received an intravenous dose (via femoral vein) of ^{14}C-labeled AIB. At different intervals up to 60 minutes after the injection, samples of maternal blood were taken (with heparinized capillary tubes) from the suborbital sinus: one placenta and one fetus were removed, with alternating uterine horns for each sampling. Radioactivity was determined in samples of plasma and whole placenta and fetus homogenates.

Transfer of AIB from the maternal circulation into the fetus follows a pattern similar to that reported for other amino acids in the rat (Fig. 1). Shortly after injection, the placenta concentrates a considerable amount of AIB compared to the fetus. At 10 minutes, the placental concentration of AIB is approximately ten times higher than the concentration in fetal tissues. The placental concentration decreased with time, but it was higher than the fetal concentration throughout the study. Concentration in the fetus changed reciprocally with concentration in placenta, reflecting the rate of placental transfer of AIB. Plasma concentration of AIB decreased steadily with time, as was expected.

Malnourished animals had the same general pattern of AIB transfer as the control rats, but there were several significant differences between these two groups. Placental AIB concentration of the experimental animals was lower than that of the control group at 10 and 20 minutes and higher than that of the controls at 40 and 60 minutes (Fig. 1A). This difference suggests a reduced uptake of AIB from the maternal blood, combined with a proportionally more reduced rate of transfer of AIB to the fetus. This imbalance between input and output would explain the relative accumulation of AIB of the malnourished placentas compared to that of the control placentas. Consistent with the reduced placental uptake and transfer of AIB, the concentration of AIB in the fetuses of the malnourished group was lower than that of the controls. Plasma concentration of AIB was similar to that of control animals at 10 minutes and increasingly higher than control values thereafter. The reduced rate of disappearance of AIB from the maternal plasma probably reflected the reduced uptake of the conceptus. At day 20 of pregnancy, the total tissue mass of the placentas and fetuses represents a considerable percentage of the total metabolically active tissue mass, and it is conceivable that a reduced uptake of any substance present in the plasma would reduce the rate of disappearance from the plasma of such a substance, in this case AIB.

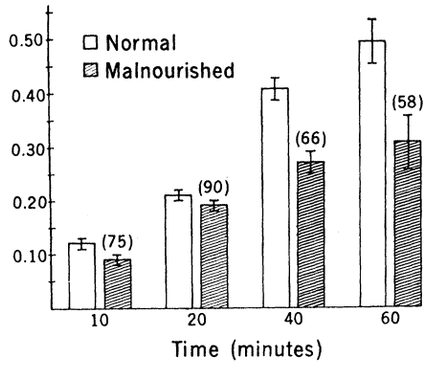

Fig. 2. The ratio of AIB concentration in the fetus to that in the placenta in normal and malnourished rats at various times after intravenous injection of AIB into the maternal circulation. Bars represent mean values ± the standard error. For each point a total of five fetuses and placentas for the normal group and four fetuses and placentas for the malnourished group were used. Analysis of statistical significance by Student's *t*-test found P values nonsignificant at 10 and 20 minutes. At 40 minutes $P < .005$ and at 60 minutes $P < .05$. The values in parentheses represent the percentage of control values.

Although these data suggest that maternal malnutrition alters placental transfer of AIB, this possibility becomes evident when the ratios of the fetal content and placental content of AIB are compared. The ratio was considerably lower in the malnourished group, reflecting the fact that after AIB is taken up from the blood it stays for a longer time in the placentas of the malnourished animals (Fig. 2). Placental transfer of amino acids is a phenomenon that is still poorly understood. It has been hypothesized that such transfer occurs in three stages: (i) active transport from the uterine blood into the placental tissue, (ii) transient storage in the placental tissue, (iii) diffusion from the placenta into the fetal blood (*7*). According to this idea, the stage most likely altered by malnutrition would be the first one, since it requires energy consumption. Nevertheless, present data demonstrating a reduced uptake and subsequent accumulation in the placenta suggest that all stages are affected and that either the second or the third stage is proportionally more affected than the first stage. Moreover, since blood perfusion was not measured in this model, it is also possible that the reduced uptake may reflect only a reduced placental perfusion of blood and that the first-stage mechanisms are functioning at a normal rate. Regardless of whether malnourished animals suffer this hemodynamic change in their placental circulation or not, the fact that AIB accumulates in the placenta suggests that the postulated second and third stages of amino acid transfer are not entirely passive phenomena. Probably energy consumption and the presence of specific proteins are required for these steps, and it is conceivable that the reduced availability of substrates imposed by maternal malnutrition may interfere with these mechanisms. Thus maternal malnutrition may prove a useful model in further elucidating the normal placental transfer of amino acids.

Related questions such as the possible influence of hemodynamic factors or the effect of maternal malnutrition in the transfer of other amino acids or other nutrients will require further studies. Regardless of the outcome of such studies, however, it seems clear that we can no longer assume that during maternal malnutrition fetal growth is solely a function of the interplay between availability of nutrients and the differences in the metabolic rate of the maternal and fetal tissues.

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References and Notes

1. J. Hammond, *Proc. Nutr. Soc.* **2**, 8 (1944).
2. J. Habicht, C. Yarbrough, A. Lechtig, R. Klein, *Nutr. Rep. Int.* **7**, 533 (1973).
3. M. Winick, in *Diagnosis and Treatment of Fetal Disorders*, K. Adamsons, Ed. (Springer-Verlag, New York, 1969), pp. 83–101; E. M. Laga, S. G. Driscoll, H. N. Munro, *Pediatrics* **50**, 33 (1972).
4. E. M. Laga, S. G. Driscoll, H. N. Munro, *Pediatrics* **50**, 24 (1972).
5. Diets with 6 percent or 27 percent casein were prepared by Nutritional Biochemicals, Cleveland, Ohio, and contained, in addition to casein, sucrose, 80 percent or 59 percent, respectively; vegetable oil, 10 percent; salt mixture, U.S.P. XIV, 4 percent; plus "vitamin diet fortification mixture" (a balanced mixture of vitamins for the rat or mouse).
6. R. A. Wapnir and C. Dierks-Ventling, *Biol. Neonate* **17**, 373 (1971).
7. L. B. Curet, *Clin. Obstet. Gynecol.* **13**, 586 (1970).

8. I thank Dr. M. Winick for his comments and M. R. Streeter for technical assistance. Supported in part by Nutrition Foundation grant 487.

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Ornithine Decarboxylase Activity: Control by Cyclic Nucleotides

Abstract. *Both exposure to cold and administration of aminophylline result in rapid increases in cyclic adenosine monophosphate (cyclic AMP) in the adrenal medulla and adrenal cortex. These increases are followed by dramatic increases in ornithine decarboxylase activity. Inhibitor studies suggest that the increase in ornithine decarboxylase activity is due to new enzyme synthesis. The data suggest that the decarboxylase activity is regulated by an increase in cyclic AMP.*

Polyamine biosynthesis is one of the earliest events that occurs during tissue growth. This increased synthesis takes the form in a variety of tissues of dramatically elevated levels of ornithine decarboxylase (ODC), the initial enzyme in the polyamine biosynthetic pathway. Activity of ODC is highest in those tissues with high rates of growth or protein synthesis. In contrast, ODC activity in static or nongrowing tissues is very low. When induced in mammalian liver by partial hepatectomy, ODC activity rises rapidly and remains elevated for 2 days (*1*). Also, ODC activity can be increased by all those hormones that affect growth processes (*2*). Following administration of inhibitors of protein synthesis, ODC activity returns to the control level, with a half-life of 10 to 20 minutes (*3*). This ability of ODC activity to fluctuate rapidly in response to the introduction or withdrawal of stimuli suggests that ODC elevation constitutes an important step in the control of cellular mechanisms. Because cyclic nucleotides are considered the second messenger in a chain of events between the receptor site on the cell membrane and the subsequent increase in biosynthetic activity, we thought that it was important to ascertain whether adenosine 3′,5′-monophosphate (cyclic AMP) might mediate the activity of ODC. We have therefore studied the relationship between cyclic AMP and ODC in the adrenal medulla of the rat. Within 30 minutes of exposure to cold (4°C), in response to cholinergic nerve impulses, the intracellular cyclic AMP increases 10- to 20-fold in the adrenal medulla of the rat (*4*). Within 2 hours this elevation disappears, and the cyclic AMP concentration returns to normal. Exposure to cold leads to the stimulation of the splanchnic nerve and results in the transynaptic induction of tyrosine hydroxylase (*4*), the regulating enzyme in catecholamine synthesis. Because of the general relation between an elevation in ODC activity and subsequent increases in biosynthetic activity, we thought that an increase in ODC activity might be intermediate between the rise in cyclic AMP in the adrenal medulla and the delayed induction of tyrosine hydroxylase. Therefore, we studied the relation between cyclic AMP and ODC in the adrenal medulla and adrenal cortex after cold exposure and in another group after aminophylline injection.

All experiments were performed with male Sprague-Dawley rats (125 to 150 g). For cyclic AMP determinations, the animals were decapitated and the adrenal glands were removed and frozen within 10 seconds. The adrenal medulla was rapidly separated from the adrenal cortex at 0° to 4°C with the aid of a dissecting microscope. Cyclic AMP was purified from the other acid-soluble nucleotides (*5*), and its concentration was determined by a protein kinase assay (*6*).

The ODC activity was measured by the release of $^{14}CO_2$ from *dl*-[1-^{14}C]-ornithine (7.66 mc/mmole, New England Nuclear) as described (*1*), with minor modifications. The buffer used was 0.05*M* sodium potassium phosphate, *p*H 7.2, containing 1 m*M* dithiothreitol (DTT). Monolaterally denervated (by splanchnicotomy) animals were purchased (Zivic-Miller, Allison Park, Pa.) and used 6 to 8 days after they underwent operation.

We substantiated the finding that within 30 minutes of subjecting the animals to cold, cyclic AMP in the adrenal medulla increased 10- to 20-fold (Fig. 1A). The cyclic AMP concentration returned to normal within 2 hours. Ornithine decarboxylase activity is elevated within 1 hour of cold exposure, becoming 10 to 20 times that of the control within 4 hours. Splanchnicotomy abolishes both the increase in

Table 1. Cyclic AMP concentration and ODC activity in the adrenal medulla and adrenal cortex of rats after cold exposure. Animals were exposed to cold (4°C, 2 hours) as described in Fig. 1. The cyclic AMP concentrations and ODC activities were determined at 0.5 and 4.5 hours, respectively, relative to the initiation of cold exposure. The experiments were performed with monolaterally denervated animals. The data are represented also relative to values obtained from control animals not subjected to cold exposure. The values are the mean ± the standard error.

Splanchnicotomy	Cold exposure	Cyclic AMP		ODC activity	
		Amount (pmole per mg of protein)	Percent of control	Amount (pmole $^{14}CO_2$/30 min per mg of protein)	Percent of control
		Medulla			
No	No	41 ± 5	100 ± 12	20 ± 4	100 ± 25
No	Yes	426 ± 22	1040 ± 55	150 ± 8	750 ± 40
Yes	No	39 ± 6	100 ± 15	24 ± 7	100 ± 29
Yes	Yes	89 ± 23	230 ± 60	43 ± 6	180 ± 25
		Cortex			
No	No	11 ± 3	100 ± 27	18 ± 3	100 ± 16
No	Yes	72 ± 19	650 ± 180	146 ± 19	810 ± 105
Yes	No	11 ± 2	100 ± 18	22 ± 4	100 ± 18
Yes	Yes	68 ± 13	620 ± 115	181 ± 12	825 ± 55