Plasma-free amino acid response to changes in dietary protein in pregnant and non-pregnant Rhesus monkeys (Macaca mulatta)

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Introduction

Intrauterine growth retardation still remains a significant clinical problem even in industrialized countries and is often associated with varying rates of perinatal morbidity and mortality (*WHO* 1970). Available data on prenatally growth retarded newborns, of whatever etiology, seem consistent with nutrient deprivation either due to lack of substrate, diminished uptake or utilization (*Katz et al.* 1975, *Gebre-Medhin et al.* 1978). However, despite extensive investigations, the mechanisms involved in intrauterine growth retardation are still unclear. Current models of experimental studies are unable to differentiate the pathophysiology of intrauterine growth retardation caused by specific nutrient deficiency from that which results from either etiological factors.

Adequate supply of amino acids is of major importance for the growing fetus and a prerequisite for the fetal synthesis of proteins. The total plasma concentration of free amino acid in the foetus is about twice the maternal concentration (*Christensen & Streicher* 1948). The concentrations of most amino acids in human maternal plasma are reduced during pregnancy compared to the non-pregnant state (*Young* 1979). Despite this, the amino acid concentration in the throphoblast is even higher compared to the maternal plasma suggesting a process involving active transport of amino acids from maternal plasma to the trophoblast and passive diffusion along a downhill gradient from the trophoblast into the foetal circulation (*Young* 1980). The transport capacity of the placenta is adaptable in vitro to changes in amino acid availability. Preincubation of placental tissue in amino acid free medium increases the cellular concentration of the non-metabolizable amino acid alpha-isobutyric acid (*Smith et al.* 1973).

In vivo studies have revealed more conflicting results. *Young & Widdowson* (1975) reported that feeding pregnant guinea pigs on a low-protein diet caused foetal growth retardation and enhanced placental transfer as well as foetal uptake of alpha-isobutyric acid. *Rosso* (1977) was unable to confirm the results of *Young & Widdowson*. *Ahokas et al.* (1981) observed that a protein and energy restricted diet in rats had a variable effect on the transplacental transfer of alpha-isobutyric acid. The present study was conducted in order to find out the effects of short-term protein restriction on free amino acid concentrations in plasma in adult non-pregnant and pregnant Rhesus monkeys. It forms part of an ongoing research project focusing on the kinetics of transplacental amino acid transfer during protein restriction.

Materials and methods.

Animals. Four pregnant and four non-pregnant healthy, well nourished Rhesus monkeys (*Macaca mulatta*) from the Primate Laboratory in Uppsala were studied. The weights of the animals at the beginning of the experiments ranged between 6.5–10.3 kg. The pregnant animals were investigated during the second half of pregnancy. All the animals were kept in individual cages. They had all shown good appetite while on their habitual diet, and did not participate in any other experiment immediately prior to or

Table 1. Composition of diet habitually served to pregnant and non-pregnant rhesus monkeys once a day.

	Total g	Protein g	Energy kcal	Carbohydrates g	Fat g
Barley	28.0	2.7	97.2	19.3	0.7
Pellets	38.0	8.7	144.0	19.0	2.3
Oats	26.0	3.0	107.0	18.2	2.4
Corn	72.0	5.7	262.0	56.4	0.9
Chick peas	21.0	4.3	63.0	12.3	1.0
Apples	150.0	0.3	87.0	21.8	0.9
Wheat	30.0.	3.0	108.0	22.5	0.4
Total		27.7	868.0	169.5	8.6

during the study. The project was approved by the Ethics Committee for the Protection of Animals at the University of Uppsala.

Diets. The monkeys were investigated during three study periods, each varying between one to two weeks: while on the habitual diet, the protein-restricted diet, and after return to the habitual diet. The food habitually consumed by the monkeys consisted of corn, pellets, and a mixture of germinated barley, oats, wheat, field peas and fruit. The amount of the habitual food served to each animal was individually decided and kept constant at an amount adequate to keep the animals in good health and stable body weight. A typical menu habitually consumed by the animals is shown in Table 1. The diet provided a monkey, weighing 7.2 kg and eating to appetite, with 3.8 g protein and 120 kcal per kg and day. Thirteen percent of the energy was provided from protein and 79 and 8 per cent from carbohydrates and fat, respectively.

The composition of the experimental diets is shown in Table 2. The experimental diet provided a monkey, weighing 7.2 kg, with 2.0 g protein and 112 kcal per kg and day. Seven per cent of the energy was provided by protein and 88 and 5 per cent from carbohydrates and fat, respectively.

The food, served once a day, was accessible to the monkeys until the next meal was served. The amount of protein and energy in the food served was calculated from standard food composition tables and information from the producers. All the monkeys had free access to water. No extra vitamins or minerals were added.

Blood sampling. Venous blood was sampled from each animal on two to four occasions, not less than 48 h apart, during each of the three study periods. The animals were fasted for 12–18 hours preceding blood sampling. Most samples were taken during the morning. All animals had been exposed to the same blood sampling procedure for

Table 2. Composition of experimental diet served to pregnant and non-pregnant rhesus monkeys once a day.

	Total g	Protein g	Energy kcal	Carbohydrates g	Fat g
Barley	28.0	2.7	97.2	19.3	0.7
Corn	105.0	8.3	382.0	83.2	1.3
Apples	400.0	0.8	232.0	58.0	2.4
Wheat	26.0.	2.6	93.9	19.5	0.3
Total		14.4	805.1	180.0	4.7

years, which was carried out by the same animal-keeper throughout the study with the animal in its cage. No restraint of the animals was needed during the blood sampling.

Analytical methods. The blood samples were immediately centrifuged and the plasma was frozen and stored at minus 70°C for later analysis. All samples were treated similarly. The concentrations of free amino acids in plasma were determined using ion exchange chromatography on a Beckman LC 200 automatic amino acid analyzer. Precipitation was achieved by adding 100 µl of 10 % sulphosalicylic acid to 400 µl of plasma before analysis.

Results

The pregnant monkeys had generally lower plasma-free amino acid concentrations, except for Tau, Gln and Met, than the non-pregnant monkeys during the first study period when both groups of monkeys were on their habitual diet (Fig. 1). The reduction in plasma concentrations of amino acids ranged between 1–73 %, while the increase in Tau, Gln and Met was of the order of 13–101 %.

Means and ± 1 SD of amino acid concentration in the pregnant and in the non-pregnant monkeys during habitual diet, while on protein restriction, and after return to the habitual diet, are shown in Figs. 2 and 3 respectively.

After introduction of the experimental diet, the pregnant monkeys displayed a further lowering of all amino acid concentrations except Gln (Fig. 2). This trend was reverted on resumption of the habitual diet in the case of Tau, Ser, Glu, Gly, Val, Ile, Phe, Orn, and Lys. The reduction in Asp, Pro, Ala, and Arg continued even after resumption of the habitual diet.

The non-pregnant monkeys on the other hand, showed an increase in the amino acid concentrations of Tau, Asp, Gln, Pro, Ala, Met, Ile, Leu, Tyr, Orn, Lys, His, and Arg while on the experimental diet (Fig. 3). These amino acids, with the exception of Gln which did not change further, continued to increase, even after resumption of the habitual diet. In contrast, the concentrations of Thr, Ser, Glu, Gly, Cit, Val, and Phe decreased when the monkeys were on the protein-restricted diet. The decrease was reverted upon resumption of the habitual diet.

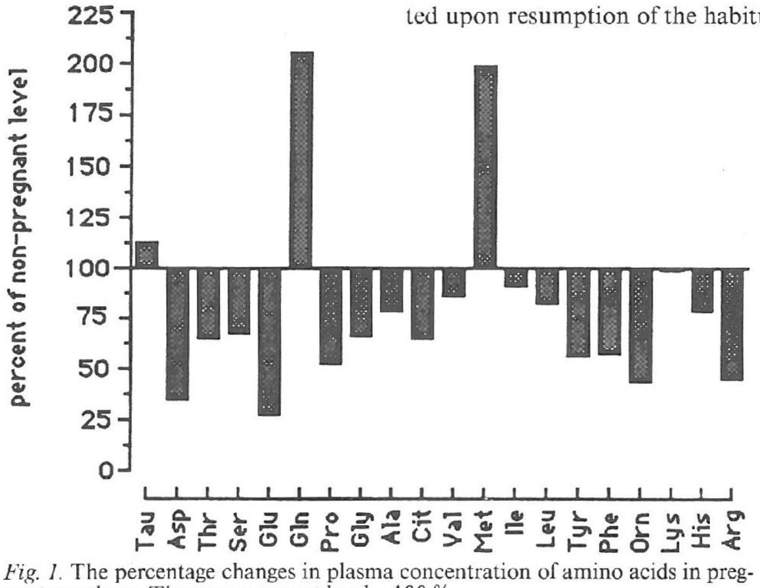

Fig. 1. The percentage changes in plasma concentration of amino acids in pregnant monkeys. The non-pregnant level = 100 %.

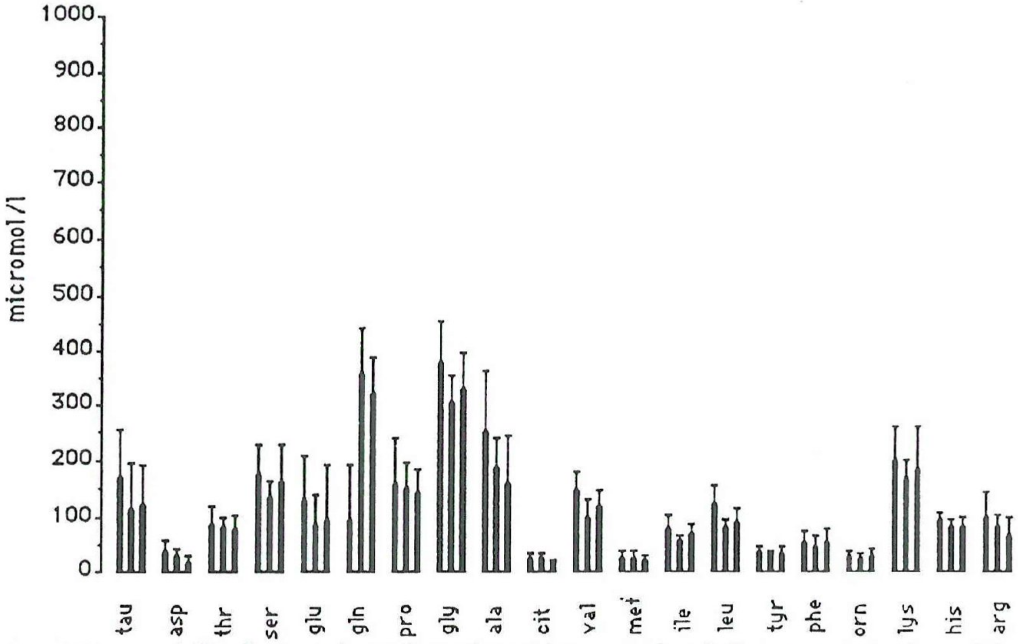

Fig. 2. Means ± 1 SD of venous plasma-free amino acid concentrations in four pregnant monkeys while on their habitual diet, during protein restriction, and after return to the habitual diet.

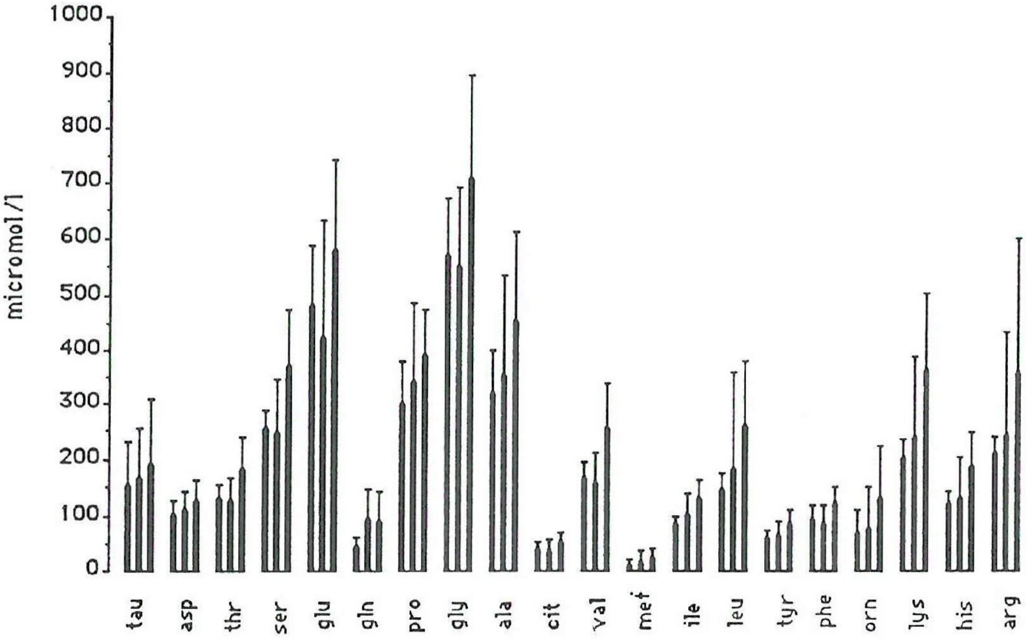

Fig. 3. Means ± 1 SD of venous plasma-free amino acid concentrations in four non-pregnant monkeys while on their habitual diet, during protein restriction, and after return to the habitual diet.

Discussion

The primary objective of the present study was to investigate the effect of short-term dietary protein restriction on the plasma-free amino acid pattern in pregnant and non-pregnant Rhesus monkeys. In vivo studies on this issue have previously given conflicting results.

The experimental diet provided the monkeys with about 50 % of the protein available in the habitual diet, with only a slight reduction of energy in this diet. In this way the proportion of energy as protein was reduced from 13 % to 7 %. As a consequence, the proportion of energy derived from carbohydrates was increased from 79 % to 88 % and that of fat was reduced from 8 % to 5 %. This implies a reduction of energy in the experimental diet amounting to about 7 % of the habitual diet. Thus, the change from habitual diet to experimental diet was mainly one of protein reduction.

Normal pregnancy is characterized by a physiological reduction of plasma-free amino acid concentrations compared to the non-pregnant state. Our findings (Fig. 1) are in accordance with results from previous studies in humans (*Young* 1979) based on other methods for amino acid analyses, as well as in non-human primates (*Kerr* 1967) using paper chromatography. Pregnancy induced changes mediated by hormones and increases in maternal vascular volume, renal clearance, as well as fetal-placental demand for nutrients are likely to lead to a reduction of the maternal amino acid concentrations compared to the non-pregnant state.

Long-standing protein malnutrition in children and in adults has previously been shown to be associated with a depression of the amino acid concentration (*Saunders et al.* 1967, *Gebre-Medhin et al.* 1978). In the present study, rapid and wide-spread changes were seen in the free amino acid concentrations within at least two weeks after the introduction of a protein reduced diet in both the pregnant and non-pregnant monkeys. This is consistent with the findings of *Snyderman et al.* (1968) of a prompt change, mostly a depression, in the level of a number of amino acids after low protein intakes in human infants.

The change in the plasma amino acid pattern induced by the experimental diet was different in the pregnant monkeys from that of the non-pregnant monkeys. The pregnant monkeys displayed a further decrease in virtually all amino acids and a more varied pattern of change after return to the habitual diet. The reduced protein intake might lead to a further reduction of the pregnancy induced changes in amino acid concentrations. After return to the habitual diet, fetal demand and active transplacental transfer of amino acids may delay the restoration of the maternal amino acid concentrations to normal pregnancy values.

Among the non-pregnant monkeys, a mixed pattern was seen after introduction of the protein restricted diet. Several amino acids, most of which belonging to the non-essential group except Met, Ile, Leu, and His, displayed an increase. A decrease was seen among both non-essential and essential amino acids. These changes resemble the results found after milder forms of protein malnutrition in humans with raised plasma concentrations of non-essential amino acids to compensate for the decrease of the essential amino acids (*Bremer et al.* 1981).

The rise in the essential amino acids Ile and Leu found in our study suggest an effect of a reduced energy intake, since these two as well as Tyr have been reported to be sensitive to changes in endogenous insulin levels. The increase in Ala and Gln observed in our study gives further evidence of an effect of reduced energy intake. It is known that Ala and Gln are released into the circulation from the muscles during fasting (*Christensen* 1982): Ala serving as substrate for the gluconeogenesis in the liver, and Gln as an important source of energy for the splancnic tissues. We know from a previous study in our Rhesus colony that an attempt to provide a protein-reduced diet was followed by a slightly reduced total energy consumption within the present experimental set-up (*Berglund et al.* 1989). Thus, our finding in the present study of a hyperaminoacidemia in the non-pregnant group while on the experimental diet might just as well have been a result of hypoinsulinemia caused by a reduced energy intake.

The source of protein in the experimental diet used in this study differed from those of the habitual diet. The pellets and the oats, which contributed 20.5 % and 10.6 % of the protein in the habitual diet, were omitted in the experimental diet. At the same time, the proportion of protein derived from corn, barley and wheat increased from 20.5 %, 9.6 %, and 10.8 % to 57.6 %, 18.1 %, and 17.6 % from the habitual to the expermental diet respectively. The elimination of chick peas resulted in an increase in the ratio of cereal proteins in the experimental diet. Cereals are rich in Met and poor in Lys, while leguminous plants, such as chick peas, are poor in Met and rich in Lys. However, it is less likely that the rise in the concentration of Met, observed among the non-pregnant monkeys while on the experimental diet, was a result of the change in source of protein since the concentration of Lys also increased during the same period.

After resumption of the habitual diet, the non-regnant monkeys showed an increase in virtually all amino acid concentrations. This rise was probably the result of a combined effect of increased availability of amino acids in the habitual food and a general increase of food intake after return to the familar food.

In conclusion, using a modern method for complete amino acid analysis, a general reduction of most plasma-free amino acids was observed during pregnancy compared to the non-pregnant state. The pregnant and non-pregnant monkeys respond differently to the protein-reduced diet, suggesting different mechanisms of coping with dietary protein restriction. The pregnant monkeys showed a further reduction of virtually all amino acids while on the protein-reduced diet, but normalized these changes after return to the habitual diet. The non-pregnant monkeys showed a decrease in both the essential and non-essential amino acids and an increase in mainly the non-essential amino acids, while on the protein-reduced diet. The decrease was reverted and the increase continued on resumption of the habitual diet. This is probably the pattern of plasma-free amino acid changes that can be expected in primate studies involving both pregnant and non-pregnant Rhesus monkeys using an isocaloric protein-reduced diet.

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Summary

The plasma free amino acid response to short-term dietary isocaloric protein reduction was studied in four non-pregnant and four pregnant rhesus monkeys (*Macaca mulatta*). Before the introduction of the protein-reduced diet, a general reduction of most plasma free amino acids was observed during pregnancy compared to the non-pregnant state. The pregnant and non-pregnant monkeys responded differently to the protein-reduced diet, suggesting different mechanisms of coping with dietary protein restriction. The pregnant monkeys showed a further reduction of virtually all amino acids while on the protein reduced diet, but normalized these changes after return to the habitual diet. The non-pregnant monkeys showed a decrease in both the essential and non-essential amino acids and an increase in mainly the non-essential amino acids, while on the protein-reduced diet. The decrease was reverted and the increase continued on resumption of the habitual diet. This is probably the pattern of plasma-free amino acid changes that can be expected in primate studies involving both pregnant and non-pregnant Rhesus monkeys using an isocaloric protein-reduced diet.

Sammanfattning

Effekten på aminosyremönstret i plasma efter en till två veckors isocalorisk proteinreducerad kost studerades hos fyra ickegravida och hos fyra gravida Rhesusapor (*Macaca mulatta*). Den tidigare kända generella reduktionen av de flesta aminosyrakoncentrationer hos gravida jämfört med icke-

gravida kunde bekräftas. De gravida och de icke-gravida apornas aminosyrakoncentrationer uppvisade olika mönster i samband med proteinreducerad kost. De gravida visade en ytterligare minskning av så gott som alla aminosyrakoncentrationer men en normalisering efter återgång till normalkost. De ickegravida aporna visade under proteinreducerad kost en minskad koncentration av både essentiella och icke-essentialla aminosyror samt en ökad koncentration av väsentligen de icke-essentiella aminosyrorna. Minskningen återhämtades och ökningen fortsatte efter återgång till normalkost. De påvisade aminosyramönstren bland både gravida och ickegravida apor är sannolikt vad man kan förvänta sig hos apor som serverats en isocalorisk proteinreducerad kost.

Yhteenveto / K. Pelkonen
Tutkimuksessa selvitettiin proteiinien vähentämisen vaikutusta plasman vapaiden aminohappojen määrään syöttämällä lyhytaikaisesti isokalorisia dieettejä neljälle kantevalle ja neljälle ei-kantavalle reesusapinalle. Kantavana olemisen havaittiin sinänsä laskevan plasman vapaitten aminohappojen määrää jo ennen proteiinikäyhän dieetin syöttämistä. Kantavien ja ei-kantavien apinoiden vaste proteiiniköyhään diettiin oli erilainen, joka vittaa siihen että niillä erilaiset mekanismit vastaavat proteiinien vähentymiseen. Kantavilla apinoilla proteiinirajoitus laski entisestään jo alentunutta plasman proteiinien määrää, mutta muutos oli palautuva, kun aloitettiin normaalidietti. Ei-kantavilla apinoilla sekä vältämättömien että ei-välttämättömien aminohappojen määra laski ja nimenomaan ei-välttämättömien aminohappojen määrä nousi syötettäessä vähäproteiinista ruokaa. Tämä lasku kääntyi nousuun ja nousu jatkui, kun palattiin tavanomaiseen ruokintaan. Tämäntapaista reaktiotapaa voidaan todennäköisesti odottaa syötettäesä kädellisiä kokeissa, joissa sekä kantaville että ei-kantaville reesusapinoille syötetään isokalorista vähäproteiinista ruokaa.

References

Ahokas RA, EB Lahaye, GD Anderson & J. Lipshitz: Effect of maternal dietary restriction on foetal growth and placental transfer of alpha-amino isobutyric acid in rats. J. Nutr. 1981, *111*, 2052–2058.

Berglund L, M Gebre-Medhin, BS Lindberg, A Lilja, H. Lundqvist & B. Långström: Placental transfer of methionine during protein restriction in the Rhesus monkey studied by positron emission tomography. Placenta 1989, *10*, 387–397.

Bremer H, M. Duran, JP Kamerling, H. Przyrembel & SK Wadman (Eds.): Disturbances in Amino Acid Metabolism: Clinical Chemistry and Diagnosis. Urban & Schwarzenberg, Baltimore-Munich 1981.

Christensen NH & JA Streicher: Association between rapid growth and elevated cell concentrations of amino acids. J. Biol. Chem. 1948, *175*, 95–101.

Christensen, HN: Interorgan amino acid nutrition. Physiol. Rev. 1982, *62*, 1193–1233.

Gebre-Medhin M, U Larsson, BS Lindblad & R Zetterström: Subclinical protein-energy malnutrition in under-privileged Ethiopian mothers and their newborn infants. Acta Paediatr. Scand. 1978, *67*, 213–217.

Katz M, GT Keusch & L Mata: Malnutrition and infection during pregnancy: Determinants of growth and development of the child. Editors' Comments. Am. J. Dis. Child. 1975, *129*, 419–420.

Kerr GR & HA Waisman: Transplacental ratios of serum free amino acids during pregnancy in the Rhesus monkey. In: Amino Acid Metabolism and Genetic variation Conference (Ed. WL Nyhan) McGraw-Hill, New York 1967, 429–437.

Rosso P: Maternal-foetal exchange during protein malnutrition in the rat. Placental transfer of alpha-amino isobutyric acid. J. Nutr. 1977, *107*, 2002–2005.

Saunders SJ, AS Truswell, GO Barbezat, W Wittman & JDL Hansen: Plasma free amino acid pattern in protein-calorie malnutrition. Reappraisal of its diagnostic value. Lancet 1967, *2*, 795–797.

Smith CH, EW Adcock III, F Teasdale, G. Meschia & FC Battaglia: Placental amino acid uptake: tissue preparation, kinetics, and preincubation effect. Am. J. Physiol. 1973, *224*, 558–564.

Snyderman SE, LE Holt, PM Norton, E Roitman & SV Phansalkar: The plasma aminogram. I. influence of the level of protein intake and a comparison of whole protein and amino acid diets. Pediatr. Res. 1968, *2*, 131–144.

World Health Organisation: The prevention of perinatal mortality and morbidity. Technical report Ser. No. 457, 1970.

Young M & EM Widdowson: The influence of diets deficient in energy, or in protein, on conceptus weight, and the placental transfer of a non-metabolisable amino acid in the guinea pig. Biol. Neonatate 1975, *27*, 184–191.

Young M: Transfer of amino acids. In: Placental transfer (Eds. BP Chamberlain & AW Wilkinson). Pitman. London 1979, 142–158.

Young M: Placental transfer. I. Small animal models. In: Animal Models in Foetal Medicine. (Ed. PW Nathaniels). Elsevier/North-Holland Biomedical Press. Amsterdam, New York, Oxford 1980, 245–272.