Protein metabolism in the mouse during pregnancy and lactation

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Protein synthesis was measured *in vivo* in the whole body and in a number of individual tissues in mice at various stages of pregnancy and lactation. The absolute rate of protein synthesis in the whole body increased from 640 mg/day in virgin mice to 1590 mg/day by day 18 of pregnancy, and to 2100 mg/day by day 15 of lactation. Large proportions of these increments were contributed by the rapidly growing foetuses and placentae in the pregnant animals and by protein synthesis in the mammary glands during lactation. In addition, a substantial stimulation of growth and protein synthesis was also observed in the liver and the gastrointestinal tract. Gastrocnemius muscle showed no changes in protein metabolism, indicating that in the well-fed mouse this tissue is not required to play a role as a protein reserve during pregnancy and lactation.

INTRODUCTION

Pregnancy and lactation greatly increase the nutrient requirements of rats and mice (for reviews, see Williamson, 1980; Baumann & Elliot, 1983; Vernon & Flint, 1984). This is met primarily by a greater food intake, supplemented by mobilization of reserves of lipid which accumulate during the first half of pregnancy and are utilized during late pregnancy and lactation. In addition there is a decrease in lipogenesis in white and brown adipose tissue, starting in late pregnancy and continuing throughout lactation (see Vernon & Flint, 1984), while thermogenesis decreases in brown adipose tissue (Trayhurn, 1985). Utilization of carbohydrates and lipids by maternal tissues is minimized in favour of the foetuses, placentae and mammary glands.

Adaptations of protein metabolism during pregnancy and lactation are less clearly defined. There is some evidence for an accumulation of carcass protein in rats during early pregnancy, and it has been suggested that this protein is subsequently used to support foetal growth in the late stages (Beaton *et al.*, 1954; Naismith & Morgan, 1976; Mayel-Afshar & Grimble, 1982, 1983). However, the quantitative importance of such changes is uncertain, and it is not known whether adaptations in the protein metabolism of maternal tissues makes a significant contribution to the extra requirement for amino acid to support foetal growth. An early study (Spray, 1950) suggested a loss of whole-body protein in rats and mice during lactation. Other studies have reported a loss of fat-free dry matter from the carcass during lactation (Moore & Brasel, 1984) and imply a loss of whole body protein (Kanto & Clawson, 1980), and there is also evidence for increased 3-methylhistidine excretion during lactation in rats, suggesting the possibility of increased turnover of muscle protein (Sainz *et al.*, 1984). On the other hand, several studies have reported that muscle or carcass protein did not change during lactation in rats (Remesar *et al.*, 1981; Naismith *et al.*, 1982; Siebrits *et al.*, 1985) and mice (Johnson, 1973) during lactation. We have therefore examined the changes in protein

synthesis and content in the whole body at different stages of pregnancy and lactation in the mouse, and analysed the contributions made individually by several major tissues.

MATERIALS AND METHODS

Animals

Adult mice (Tuck's No. 1 strain; A. Tuck and Sons, Rayleigh, Essex, U.K.) were housed at 22–24 °C, with a 12 h-light/12 h-dark cycle (light 07:00 to 19:00 h). Animals had free access to water and food. Virgin females (30–35 g) were mated with males of the same strain on a one-to-one basis, and the day of appearance of a vaginal plug was taken as day 1 of pregnancy. Pregnant mice were housed in groups until 2 days before parturition, after which they were caged individually. On the day of parturition (day 1 of lactation), litters were decreased to ten pups. To minimize diurnal variation, all experiments involving measurement of protein synthesis were performed between 10:00 and 12:00 h. Plasma-volume measurements were made between 12:00 and 14:00 h. Protein synthesis rates *in vivo* were determined on days 10 and 18 of pregnancy (gestation period 19.5 days), on days 3 and 15 of lactation, and in virgin mice of the same age.

Measurement of protein synthesis

Protein synthesis was measured *in vivo* by using the 'high dose' method described by Garlick *et al.* (1980). Mice were injected through the lateral tail vein with a mixture of unlabelled and L-[4-³H]phenylalanine (Amersham International, Amersham, Bucks., U.K.) such that 150 µmol and 100 µCi were administered in 1 ml per 100 g body wt. In experiments where the maternal body, foetuses, placentae and mammary glands were studied, two groups of mice were used for each measurement, one group killed 2 min and the other 10 min after the administration of the label. This enabled a mean value for the specific radioactivity of the free [³H]phenylalanine during the labelling period in each tissue to be determined

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and used in the calculation of protein-synthesis rates by the method of Garlick *et al.* (1980). For the liver, gastrointestinal tract and gastrocnemius muscle, however, the specific radioactivity of [^3H]phenylalanine in the tissue free pool during the labelling period has been found to change under these conditions to such a small extent that a single measurement at 10 min constitutes a sufficiently accurate estimate of that throughout the whole period (Goldspink & Kelly, 1984; Lewis *et al.*, 1984; Goldspink *et al.*, 1984).

Animals were killed by cervical dislocation, and tissues were rapidly removed and frozen in liquid N_2 . When synthesis rates in the whole body were measured, the intact body was placed in ice-cold 0.9% NaCl for the removal of the foetuses, placentae and mammary glands. The gut contents were removed by flushing with cold 0.9% NaCl and the remaining carcass was frozen in liquid N_2 . These carcasses were then freeze-dried and ground in a mincer before analysis. Individual frozen tissues were prepared for analysis by grinding under liquid N_2 in a pestle and mortar. All the foetuses from each pregnant mouse, together with the placentae, were treated as a single tissue, as was the entire gastrointestinal tract, inclusive of the stomach. Weighed samples of tissue were thawed and deproteinized in ice-cold 2% (v/v) HClO_4 .

Processing of the samples and measurement of the specific radioactivity of the protein-bound and free [^3H]phenylalanine and of the tissue protein content were as described by Garlick *et al.* (1980). The fractional rate of protein synthesis was calculated as described by these authors.

Determination of the ratio of plasma protein synthesis to total liver protein synthesis

Mice were each injected intravenously with 0.2 ml of 0.9% NaCl containing 5 μCi of L-[1- ^{14}C]leucine (Amersham International) 2 h before the administration of the flooding dose of [^3H]phenylalanine described in the preceding section. The mice were killed 10 min after the second injection, and blood was taken from the thoracic cavity before other tissues were removed as usual. After the injection of a tracer dose of [1- ^{14}C]leucine, secretion of labelled plasma proteins begins after 15 min and is essentially complete after 2 h (Morgan & Peters, 1971; Scornik & Botbol, 1976). The proportion of total liver protein synthesis devoted to the production of plasma proteins was calculated from the incorporation of [^{14}C]leucine into plasma and liver proteins, as the ratio: (total d.p.m. in plasma protein)/(total d.p.m. in liver protein + total d.p.m. in plasma protein).

To measure the radioactivity incorporated into plasma proteins, protein was precipitated from 0.025 or 0.05 ml samples of plasma with 0.25 ml of 10% (w/v) trichloroacetic acid. The precipitates were washed with 3 $\times$ 0.5 ml of 5% trichloroacetic acid, dissolved in 0.25 ml of 0.3 M-NaOH and re-precipitated with 0.5 ml of 10% trichloroacetic acid. The precipitates were washed with 1 ml of 5% trichloroacetic acid and redissolved in 0.2 ml of 0.3 M-NaOH for determination of radioactivity. Weighed samples of liver (100–150 mg) were added to 2 ml of 2% (v/v) HClO_4 and taken through a procedure of washing and solubilization similar to that for the plasma proteins. ^{14}C radioactivity was determined in a Beckman LS 2800 liquid-scintillation spectrometer, by using a dual-label program for ^3H and ^{14}C .

To calculate the total radioactivity present in liver or plasma protein from the data obtained as described above, it was necessary to know the total liver mass and the plasma volume of each animal. Plasma volumes were measured in a parallel group of animals by the method of Kernoff *et al.* (1971), based on the dilution of a known amount of ^{125}I -labelled human albumin (Amersham International), injected through the lateral tail vein. Each mouse received 0.1 μCi of this material in 0.2 ml of 0.9% NaCl, and was killed after 10 min. The blood was rapidly removed from the thoracic cavity and plasma prepared. To ensure that only protein-bound radioactivity was measured, protein was precipitated from the plasma samples and also from the injection solution (in the presence of 'carrier' plasma). Radioactivity in the precipitates was measured with a Multigamma II instrument (LKB); 96–98% of the radioactivity was found to be protein-bound. Plasma volumes were expressed per g body wt. By using a mean value, the plasma volumes of the mice used for protein-synthesis measurements could then be calculated.

The validity of the ^{125}I -albumin dilution method for measuring plasma volume depends on the assumption that the rate of disappearance of the labelled albumin from the plasma was negligible over the 10 min period (Scornik & Botbol, 1976). It is also necessary that during this time the labelled albumin should not cross, or be metabolized by, the placenta. Several reports have indicated that only a very small proportion of maternal plasma proteins crosses the placenta (Gitlin & Koch, 1968; Masters *et al.*, 1969). It was therefore assumed in our experiments that neither the injected ^{125}I -albumin, nor the ^{14}C -labelled plasma proteins secreted from the liver, were lost from the maternal into the foetal circulation.

RESULTS AND DISCUSSION

In this study we have investigated changes in the amounts and rates of synthesis of protein in some of the major organs of the body during pregnancy and lactation in the mouse, in order to assess the relative contributions of each organ to the effects on the body as a whole. Table 1 shows an analysis of the overall changes in protein mass of the maternal body and of the foetuses, placentae and mammary glands growing within it. The total body protein content increased from 3205 to 4530 mg by day 18 of pregnancy. Most of this increase (82%) was due to the development of the foetuses and placentae, which showed a 25-fold increase in protein mass between days 10 and 18 of pregnancy. The remainder was mostly due to the growth of the mammary glands. Naismith & Morgan (1976) have suggested that there is an anabolic phase of protein metabolism in the maternal body during the first two-thirds of pregnancy, followed by a period of mobilization of maternal body protein for use by the growing foetuses. The measurements of protein content in the maternal carcass in the present study suggest that such a cycle is of little importance in the mouse fed *ad libitum*. Furthermore, we found no evidence of any loss of whole-body protein during lactation. This is contrary to the findings of Spray (1950), who reported a substantial fall in whole-body protein in mice during lactation, but is in agreement with those of Johnson (1973), who found an increase in whole-body N.

Table 2 shows the effects of pregnancy and lactation

Table 1. Changes in protein mass of the maternal body, foetus and placenta and mammary gland during pregnancy and lactation

Values are means $\pm$ S.E.M. of data from ten animals per group. The mass of mammary tissue was negligible in the virgin animals. The percentage contribution of each compartment to total body protein mass is shown in parentheses. Significance of differences from virgin group: * $P < 0.001$. Significance of differences from 10-day-pregnant group: † $P < 0.001$

Animals		Protein mass (mg)		
		Maternal body	Foetuses + placenta	Mammary glands
Virgin		3205 $\pm$ 132 (100)	—	—
Pregnant:	10 days	3389 $\pm$ 151 (97)	42.6 $\pm$ 3.8 (1)	52.2 $\pm$ 4.9 (1)
	18 days	3313 $\pm$ 169 (73)	1081 $\pm$ 115† (24)	136 $\pm$ 11† (3)
Lactating:	3 days	4201 $\pm$ 222* (94)	—	263 $\pm$ 12† (6)
	15 days	4224 $\pm$ 236* (89)	—	538 $\pm$ 34† (11)

Table 2. Fractional and absolute rates of protein synthesis in the maternal body, foetuses and placenta and mammary glands of the mouse during pregnancy and lactation

Values are means $\pm$ S.E.M. of data from six animals per group: n.d., not determined. The percentage contribution of each compartment to the total protein synthesis of the whole body is shown in parentheses with the absolute rates. Significance of differences from virgin group: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Significance of differences from 10-day-pregnant group: † $P < 0.001$.

Animals		Rate of protein synthesis					
		Fractional (%/day)			Absolute (mg of protein synthesized/day)		
		Maternal body	Foetuses plus placenta	Mammary gland	Maternal body	Foetuses plus placenta	Mammary gland
Virgin		20.9 $\pm$ 0.9	—	n.d.	641 $\pm$ 43 (100)	—	—
Pregnant:	10 days	23.7 $\pm$ 0.5	102 $\pm$ 4	41.2 $\pm$ 2.2	821 $\pm$ 51* (93)	39.5 $\pm$ 1.2 (4)	24.8 $\pm$ 3.5 (3)
	18 days	26.2 $\pm$ 0.9**	58.0 $\pm$ 3†	82.7 $\pm$ 4.0†	938 $\pm$ 84** (59)	543 $\pm$ 52† (34)	113 $\pm$ 13† (7)
Lactating:	3 days	27.5 $\pm$ 1.4***	—	143 $\pm$ 9†	1154 $\pm$ 131*** (75)	—	386 $\pm$ 35† (25)
	15 days	28.2 $\pm$ 1.5***	—	150 $\pm$ 16†	1297 $\pm$ 109*** (62)	—	799 $\pm$ 102† (38)

on the rates of protein synthesis in the whole body and in the foetuses plus placenta and mammary glands. The results are expressed in two ways, firstly as fractional rates (i.e. the percentage of the total tissue protein pool that is synthesized per day) and secondly as absolute rates (the total amount of protein synthesized per day by that tissue). When the maternal tissues alone were examined, the fractional rate of protein synthesis was found to increase only slightly over the period of pregnancy and lactation. Since the fractional accretion rate of maternal body protein (Table 1) was extremely slow relative to the protein-synthesis rate, it follows that the rate of degradation must have risen roughly in line with the synthesis rate. There was a pronounced activation of protein synthesis in the mammary gland. The fractional rate doubled between days 10 and 18 of pregnancy and had nearly doubled again by day 3 of lactation. During lactation it is likely that much of the protein synthesized within the gland is secreted, since Rosen *et al.* (1980) have estimated that casein and α -lactalbumin mRNAs comprise 80% of total mammary mRNA after 10 days of lactation in the rat. However, even at 10 and 18 days of pregnancy high fractional synthesis rates were found (Table 2). Before parturition,

casein mRNA constitutes a lower proportion of total mRNA (Rosen & Barker, 1976), so it is likely that much of the stimulation of protein synthesis in the mammary gland of pregnant animals represents cellular proteins, and is associated with the development and proliferation of the tissue. However, by late pregnancy in the rat, a substantial amount of casein mRNA has accumulated (Rosen & Barker, 1976), and its presence in polysomes suggests that translation of milk proteins is already occurring (Rosen *et al.*, 1978). One possibility is that newly synthesized milk proteins are degraded in the pregnant animal, as suggested by the work of Hasan *et al.* (1982) on isolated explants from mammary glands of pregnant rabbits.

Values for the fractional synthesis rate in the mammary gland have been published for rats at peak lactation (Sampson & Jansen, 1984a, 1985; Sampson *et al.*, 1986; Sainz *et al.*, 1986), and, although they were slightly lower than the rates that we observe here in the mouse at peak lactation, they also indicate high protein-synthetic activity. Jansen and colleagues have found the elevated protein-synthesis rates during lactation to be very sensitive to the supply and protein quality of the diet (reviewed by Sampson & Jansen, 1984b).

The fractional rate of protein synthesis in the foetus and placenta was, as expected, high relative to that of most adult tissues (Table 2). The rate in the 18-day-pregnant animals was lower than that observed at mid-pregnancy. The data were similar to those obtained by Goldspink & Kelly (1984), who also used the 'high dose' method for measuring protein synthesis in rat foetuses. In a preliminary experiment they showed that the specific radioactivity of the free pool of amino acid in the foetal tissue increased over the first 2 min after injection, and then remained constant for the ensuing 8 min, indicating that the presence of the placental barrier between foetal and maternal circulation does not cause a serious delay in the uptake of the radioactive precursor by the foetal tissues. In earlier studies (Mayel-Afshar & Grimble, 1982, 1983) the fractional synthesis rate in foetus and placenta in the rat was determined by constant infusion of [14 C]tyrosine for 6 h, but the values obtained were lower than those found by Goldspink & Kelly (1984) and ourselves (Table 2). This discrepancy may be due to the different methods used, since prolonged infusion techniques involving tracer doses of radioisotope can be unsuitable for tissues with high turnover rates (McNurlan *et al.*, 1979; McNurlan & Garlick, 1981), and may also introduce errors owing to metabolism of the tracer by the placenta before it reaches the foetal circulation.

The measurements that we have made of protein mass and fractional synthesis rate enable us to calculate the absolute rates of protein synthesis by the maternal body, the foetuses plus placenta and the mammary glands at different stages of pregnancy and lactation, and to evaluate their contributions to the total protein synthesis by the whole body (Table 2). By 18 days of pregnancy the rate of protein synthesis of the whole body had increased from 640 to 1590 mg/day. Of this increment 57% could be attributed to the foetuses and placenta and a further 12% to protein synthesis in the mammary gland (Table 2). Even though the fractional synthesis of the foetus plus placenta decreased between day 10 and day 18 of pregnancy, the absolute rate still rose more than 10-fold over this period, and by 18 days accounted for 34% of whole-body synthesis.

By 15 days of lactation the rate of protein synthesis of the whole body had increased to 2100 mg/day. A large proportion of the increase over the virgin value (55%) was due to synthesis by the mammary glands; by this stage the absolute rate of protein synthesis of the mammary gland exceeded that of the whole body of virgin mice. This tissue comprised 11% of whole-body protein mass, but was responsible for 38% of protein synthesis. With the mammary gland synthesizing 800 mg of protein/day, the potential capacity of the maternal body (protein mass 4200 mg) to provide a protein reserve seems somewhat insignificant; clearly, increased food intake is the major source of substrate. In this strain of mice, the food intake has been shown to rise to 4 times the virgin value by peak lactation (C. H. Knight, unpublished work).

Growth and protein synthesis in individual tissues of the maternal body

Table 3 shows the changes in protein mass of three major maternal tissues, the liver, the gastrointestinal tract and, as an example of skeletal muscle, the gastrocnemius, during pregnancy and lactation. The gastrointestinal tract included all regions from the

Table 3. Changes in protein mass of liver, gastrointestinal tract and skeletal muscle during pregnancy and lactation

Values are means $\pm$ S.E.M. of observations on six to eight animals per group. Significance of differences from virgin group: * $P < 0.05$, ** $P < 0.001$.

Animals		Protein mass (mg)		
		Liver	Gastro-intestinal tract	Gastrocnemius muscle
Virgin		218 $\pm$ 10	218 $\pm$ 8	45.6 $\pm$ 1.3
Pregnant:	10 days	256 $\pm$ 15	259 $\pm$ 20*	47.6 $\pm$ 1.2
	14 days	361 $\pm$ 9*	233 $\pm$ 20	50.3 $\pm$ 3.1
	18 days	406 $\pm$ 30**	303 $\pm$ 20**	49.6 $\pm$ 1.5
Lactating:	3 days	487 $\pm$ 28**	317 $\pm$ 11**	50.3 $\pm$ 1.8*
	9 days	441 $\pm$ 18**	379 $\pm$ 33**	49.2 $\pm$ 1.4
	15 days	606 $\pm$ 57**	537 $\pm$ 17**	51.3 $\pm$ 1.3*

stomach to the rectum, flushed out to remove the contents. Throughout pregnancy and lactation both the liver and gastrointestinal tract showed marked increases in mass, whereas the growth of skeletal muscle was slow, as would be expected in virgin control mice of the same age, which grow at a rate of about 0.4%/day. Thus by 15 days of lactation the proportions of body weight attributable to liver (8.3%) and gastrointestinal tract (12%) were considerably larger than those in virgin controls (4.9% and 7.3% respectively). The accelerated growth rate of the liver was maintained consistently over the whole period. Increases in the weight and protein content of the liver during pregnancy and lactation have been shown previously in rats (Chatwin *et al.*, 1969; Campbell *et al.*, 1974; Remesar *et al.*, 1981; Mayel-Afshar & Grimble, 1982; Siebrits *et al.*, 1985). The protein mass of the gastrointestinal tract also grew steadily throughout pregnancy and early lactation, but showed a particularly marked increase as lactation approached its peak at 15 days (Table 3). Although other workers have not in general studied the gastrointestinal tract as a whole, the data obtained here are consistent with reports of changes in the mass of specific regions of the rat intestine during pregnancy and lactation (Fell *et al.*, 1963; Chatwin *et al.*, 1969; Craft, 1970; Burdett & Reek, 1979; Palmer & Rolls, 1980; Remesar *et al.*, 1981). The increase in the mass between 9 and 15 days of lactation coincides with a large increase in food intake over this period. Campbell & Fell (1964) found that the enlargement of the small intestine and caecum normally observed in lactating mice fed *ad libitum* could be prevented by restricting the food intake to the amount consumed by similar animals at late pregnancy.

The gastrocnemius muscle, unlike the liver and the gastrointestinal tract, did not appear to change its growth pattern during pregnancy and lactation (Table 3). The rates of protein synthesis in this tissue (Table 4) were also unaltered throughout the period of study, and were similar to those measured in muscles of comparable fibre composition in adult rats (Lewis *et al.*, 1984). The findings of Mayel-Afshar & Grimble (1982) suggested the possibility of a small increase in protein synthesis in skeletal muscle during early and mid-pregnancy in rats, though this was not significant statistically. This trend

Table 4. Rates of protein synthesis in the liver, gastrointestinal tract and skeletal muscle of the mouse during pregnancy and lactation

Values are mean $\pm$ S.E.M. of data from six to eight animals per group. Significance of differences from virgin group: * $P < 0.05$; ** $P < 0.01$.

Animals	Rate of protein synthesis					
	Liver		Gastrointestinal tract		Gastrocnemius muscle	
	Fractional (%/day)	Absolute (mg/day)	Fractional (%/day)	Absolute (mg/day)	Fractional (%/day)	Absolute (mg/day)
Virgin	64.3 $\pm$ 1.6	141 $\pm$ 8	84 $\pm$ 3	183 $\pm$ 10	4.88 $\pm$ 0.19	2.23 $\pm$ 0.13
Pregnant:						
10 days	65.6 $\pm$ 4.0	169 $\pm$ 16	100 $\pm$ 7*	269 $\pm$ 35**	4.96 $\pm$ 0.25	2.36 $\pm$ 0.11
18 days	112 $\pm$ 5.3**	466 $\pm$ 43**	110 $\pm$ 6**	335 $\pm$ 32**	4.89 $\pm$ 0.33	2.44 $\pm$ 0.20
Lactating:						
3 days	78.1 $\pm$ 4.6**	387 $\pm$ 41**	90 $\pm$ 7	285 $\pm$ 21**	4.55 $\pm$ 0.27	2.28 $\pm$ 0.13
15 days	73.4 $\pm$ 2.4**	439 $\pm$ 28**	95 $\pm$ 4*	511 $\pm$ 33**	4.51 $\pm$ 0.13	2.31 $\pm$ 0.002

Table 5. Estimated synthesis rates of hepatic cellular proteins and of plasma proteins by the liver of pregnant and lactating mice

Values are means $\pm$ S.E.M. of data from six to eight animals per group. Significance of differences from virgin controls: * $P < 0.02$; ** $P < 0.01$. Animals were each injected with a tracer dose (5 μ Ci) of L-[1- 14 C]leucine 2 h before receiving the large dose of L-[4- 3 H]phenylalanine, as described in the Materials and methods section. Total protein-synthesis rates were obtained from the incorporation of [3 H]phenylalanine, and are given in Table 4. The proportion of liver protein synthesis devoted to the production of plasma proteins was estimated from the incorporation of 14 C radioactivity into plasma and liver protein, as described in the Materials and methods section. These calculations required knowledge of the plasma volumes in pregnant and lactating mice. These were estimated in separate groups of animals by measuring the dilution of 125 I-albumin over 5 min after intravenous injection, as described in the Materials and methods section. The values obtained for plasma volume, as ml/100 g body wt., were: virgin, 6.9 $\pm$ 0.3; 10-day pregnant, 7.9 $\pm$ 0.5; 18-day pregnant 8.2 $\pm$ 0.2; 3-day lactating, 8.2 $\pm$ 0.5; 15-day lactating, 8.6 $\pm$ 0.5.

Animals		Plasma protein synthesis	Fractional rate of synthesis of hepatic cellular proteins (%/day)
		Total hepatic protein synthesis	
Virgin		0.26±0.02	48.2±1.7
Pregnant:	10 days	0.31±0.02	44.2±3.2
	18 days	0.38±0.04*	75.2±7.9**
Lactating:	3 days	0.24±0.01	59.3±3.5**
	15 days	0.26±0.01	54.4±2.0*

was not seen in the present study, and our results are not consistent with a role for muscle as a protein reserve during normal pregnancy in mice, as suggested by Naismith & Morgan (1976) for rats. Our failure to detect any appreciable change in muscle protein synthesis during lactation is in agreement with the observations by Siebrits *et al.* (1985), who found only a very small decrease at 12 days of lactation in rats. Taking our data for protein synthesis (Table 4) and protein mass (Table 3) together, we can also conclude that protein degradation in muscle was not affected by pregnancy and lactation. Our results, however, do not exclude a potential role for muscle protein breakdown in supporting foetal growth during abnormal nutritional stress.

Table 4 shows a small increase in the fractional rate of protein synthesis in the gastrointestinal tract during the latter half of pregnancy. The rate appeared to fall after parturition, but still remained slightly elevated over that in virgin animals during lactation. Analysis of the gastrointestinal tract as a whole prevents us from drawing conclusions about the responses of the individual regions, but calculation of the absolute rates indicates that the overall contribution of the gastrointestinal tract to

whole-body protein synthesis is considerably increased in pregnant and lactating mice.

Of the three tissues examined, the liver showed by far the most pronounced changes in protein synthesis, particularly during the late stage of pregnancy (Table 4). It is possible that increased food intake may be at least partly responsible for the more modest elevation of protein synthesis maintained during lactation, but this is unlikely to explain the very high rate in the 18-day-pregnant animals. Two important factors, however, could be the expansion of the plasma volume and the increased rate of turnover of plasma proteins that occur by late pregnancy (Brown & Pike, 1960; Masters *et al.*, 1969; Rosso & Streeter, 1979). Plasma protein synthesis accounts for about 30% of the overall protein-synthetic effort of the liver, and a changed need for production and maintenance of the pool of plasma proteins could well have an impact on the overall rate of hepatic protein synthesis. In order to investigate this, we have attempted to partition the values for total liver protein synthesis between production of plasma proteins and synthesis of hepatic cellular proteins. This was done by measuring the distribution of radioactivity from a tracer dose of

[1-¹⁴C]leucine between the total plasma proteins and total liver proteins 2 h after injection of the label, as described in the Materials and methods section. This method does not allow for the loss of rapidly labelled proteins in either liver or plasma during the 2 h labelling period. However, a study by Scornik & Botbol (1976) indicated that this procedure gave a very similar value to that obtained by a more accurate determination which took decay of rapidly turning-over proteins into account. In a separate experiment we determined the plasma volumes, and found these to be expanded in all groups of pregnant and lactating mice (see legend to Table 5). Table 5 shows that, in the 18-day-pregnant mice, plasma protein production constituted a considerably higher proportion of total liver protein synthesis than in the other groups. In the lactating animals, the proportion was identical with that in the virgin controls, indicating that during lactation the stimulation of protein synthesis was similar for retained and for export proteins. Knowledge of values for plasma protein synthesis as a proportion of the total allowed us to calculate the fractional rates of synthesis of hepatic cellular proteins, and these estimates are also shown in Table 5. It is clear that in the 18-day-pregnant mice the disproportionate increase in the synthesis of plasma proteins was not sufficient to account for the entire rise in overall liver protein synthesis shown in Table 4. There was, in addition, a pronounced increase in the rate of synthesis of hepatic cellular proteins, and a somewhat lesser stimulation was maintained into the period of lactation. The data obtained in this work are somewhat different from those of Mayel-Afshar & Grimble (1982), who used a constant infusion of L-[U-¹⁴C]tyrosine over 6 h to measure protein synthesis during pregnancy. This is a method that measures mainly the synthesis of hepatic cellular proteins, since much of the labelled secreted protein leaves the liver during the 6 h period. Mayel-Afshar & Grimble (1982) obtained values of around 50%/day, with little consistent change throughout pregnancy. However, other workers have found that rates of protein synthesis in the liver determined by the constant-infusion method tend to differ from values obtained by other methods (McNurlan *et al.*, 1979; McNurlan & Garlick, 1981), probably because of the recycling of label back into the precursor pool. Another group of workers (Siebrits *et al.*, 1985), who, like ourselves, used the method of Garlick *et al.* (1980) to measure protein synthesis, observed a rather more pronounced stimulation of total protein synthesis in the livers of lactating rats.

Successive measurements of hepatic protein mass (Table 3) have allowed us to make approximate estimates of the fractional rate of growth. Although this was increased in the pregnant and lactating animals, the maximum rate achieved (6%/day) was still very low in comparison with the fractional rate of synthesis of hepatic cellular proteins (Table 5). Thus at all stages the rate of hepatic proteolysis must have been very close to the protein-synthesis rate. This leads to the conclusion that the rate of hepatic protein degradation is also elevated in late pregnancy, and, to a lesser extent, during lactation.

General conclusions

Pregnancy and lactation markedly increase the rate of whole-body protein synthesis in the mouse. This is

mainly due to the rapid rates of synthesis in the foetuses and placentae during pregnancy and in the mammary glands during lactation. The absolute rates of protein synthesis eventually achieved in these tissues are so large that protein reserves in the maternal body are unlikely to make a significant contribution to sustaining them. Moreover, there was no evidence for a fall in protein synthesis, relative to that in virgin controls, in any of the maternal tissues examined, at any stage. Muscle showed little change in protein synthesis, or, by deduction, in protein degradation. Protein-synthesis rates in the liver and the gastrointestinal tract were actually increased, and probably account for most of the increase in maternal body protein synthesis during the reproductive cycle. Protein synthesis accounts for about 10% of total energy expenditure in rats (Garlick, 1986), hence the doubling of the rate of protein synthesis in the maternal body during lactation is likely to result in a substantial increase in the maintenance energy requirements of the animal at a time when other energy-expending processes, such as lipogenesis in adipose tissue (Vernon & Flint, 1983) and thermogenesis in brown adipose tissue (Trayhurn, 1985), are decreased. It has been claimed that in the rat there is a decrease in the maintenance energy requirements (i.e. for functions other than milk production) during lactation, and that this is due in part to decreased protein turnover (Roberts & Coward, 1984). Clearly, in mice decreased protein turnover makes no contribution to any increase in metabolic efficiency during lactation, but the process may, through the concomitant production of heat, contribute to the decrease in thermogenesis in brown adipose tissue.

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