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Protein turnover and growth in the whole body, liver and kidney of the rat from the foetus to senility

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1. Changes in the growth and protein turnover (measured *in vivo*) of the rat liver, kidney and whole body were studied between 16 days of life *in utero* and 105 weeks *post partum*. 2. Tissue and whole-body growth were related to changes in both cellular hyperplasia (i.e. changes in DNA) and hypertrophy (protein/DNA values) and to the protein composition within the enlarging tissue mass. 3. The suitability of using a single large dose of phenylalanine for measuring the rates of protein synthesis during both pre- and post-natal life was established. The declining growth rates in the whole animal and the two visceral tissues were then explained by developmental changes in the fractional rates of protein synthesis and breakdown, turnover rates being age-for-age higher in the liver than in the kidney, which in turn were higher than those measured in the whole animal. 4. The declining fractional rates of synthesis in both tissues and the whole body with increasing age were related to changes in the tissues' ribosomal capacity and activity. The fall in the hepatic rate between 18 and 20 days of foetal life (from 134 to 98% per day) corresponded to a decrease in both the ribosomal capacity and the rate of synthesis per ribosome. No significant changes in any of these parameters were, however, found in the liver between weaning (3 weeks) and senility (105 weeks). In contrast, the fractional synthetic (and degradative) rates progressively declined in the kidney (from 95 to 24% per day) and whole body (from 70 to 11% per day) throughout both pre- and post-natal life, mainly as a consequence of a progressive decline in the ribosomal capacity, but with some fall in the ribosomal activity also during foetal life. 5. The age-related contributions of these visceral tissues to the total amount of protein synthesized per day by the whole animal were determined. The renal contribution remained fairly constant at 1.6–2.9%, whereas the hepatic contribution declined from 56 to 11%, with increasing age. 6. Approximate steady-state conditions were reached at, and between, 44 and 105 weeks *post partum*, the half-life values of mixed whole-body, kidney and liver proteins being 6.4, 3.0 and 1.5 days, respectively, at 105 weeks.

Few studies have described the normal patterns of growth and related changes in protein turnover in the major tissues of the body throughout the entire lifespan. This is perhaps surprising when so much interest has been expressed in understanding the physiological and biochemical basis behind the plastic growth of many tissues (Munro, 1970; Goss, 1972; Goldspink, 1974; Goldberg & St. John, 1976; Waterlow *et al.*, 1978, Pette, 1980; Tanner, 1981). Most investigations so far have been performed on isolated cells or on tissues of animals at one or a few ages of post-natal life. The scant information on protein turnover at the extremities of life can in part be explained by the poor availability of senile animals from commercial breeders and technical difficulties in working with small quantities of foetal tissue. However, even within the areas of post-natal life that have been more extensively studied, little attention has been paid to using the same techniques for measuring the various parameters associated with growth. Indeed, all too often information has been drawn

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together from different experiments and studies, generally ignoring differences in growth rates between the sexes and different strains of animals within, or between, species. Such differences in animal strains and their state of nutrition (Waterlow & Stephen, 1968; Waterlow *et al.*, 1978), diurnal and seasonal variations in measurements relating to growth (Wurtman, 1970; Rebolledo & Gagliardino, 1971; Garlick *et al.*, 1973) are but a few of the important factors which have often been poorly controlled in many of the earlier studies.

In the present investigation emphasis has been placed on studying the growth patterns, changes in protein turnover and nucleic acid concentrations in the whole animal and two of its visceral tissues, from the foetus through to old age. Throughout, the same techniques were rigorously applied, with measurements made at the same time of day on one strain and sex of albino rats (Charles River, CD), which had been maintained on the same diet and housed under identical conditions of heating and lighting. In this way it has been possible to assess the contribution that individual tissues make towards the growth of the whole animal throughout most of its lifespan (see also Lewis *et al.*, 1984; Goldspink *et al.*, 1984).

Methods

Throughout this study, male albino rats of the CD strain were used from our own breeding colony, stock having originally been derived from Charles River (U.K.) Ltd. (Manston, Kent, U.K.). Rats were housed in small groups in rooms controlled for temperature (22°C), and having a daily photoperiod of 12h light between 06:00 and 18:00h. All animals had free access to water and were fed *ad libitum* on a modified laboratory cubed diet (Diet 41B; Bruce & Parkes, 1949) containing a minimum of 21% (w/w) protein. Pregnant female rats (270 ± 10g) from the same colony were used for studies on foetal tissues. To minimize variations in the number and weights of the foetuses during such pregnancies, only females which had littered once previously, and had ceased to lactate 2 weeks before re-mating, were served overnight by males (i.e. a 12h mating period). The presence of sperm in vaginal smears taken between 09:00h and 10:00h the next day was taken to signify the commencement of pregnancy. Foetal tissues were subsequently taken at 16, 18 and 20 days of gestation. All experimental procedures were standardized and performed between 09:00h and 12:00h to minimize possible diurnal variations.

Protein synthesis was measured *in vivo* after an intravenous injection of a large dose of phenylalanine to flood the precursor pool (Garlick *et al.*, 1980). This injection was administered to each rat via a lateral tail vein and contained 150μmol of the amino acid, including 65μCi of L-[4-^{3}H]phenylalanine (sp. radioactivity 24Ci/mmol; from The Radiochemical Centre, Amersham, Bucks., U.K.), in 1ml of 0.9% NaCl per 100g body wt. Pregnant rats were treated similarly. In preliminary studies rats (approx. 200g males, or 20-day-pregnant females) were initially decapitated 2.5, 5, 10, 20 or 30min after injection, and blood was collected over the following 10s. The time-related changes in the specific radioactivity of phenylalanine in the tissue pool(s) relative to the plasma (both maternal and foetal, where appropriate) were measured to enable us to determine an appropriate time for measuring protein synthesis. This was found to be 10min after the injection (see below). In all of these studies the abdominal cavity was rapidly opened immediately after decapitation and the whole animal completely immersed in ice-cold NaCl. The chilled liver and kidney were immediately removed and frozen in liquid N_2. From each pregnant rat three foetuses were removed at random from the chilled uterus, and their abdominal cavities in turn were rapidly opened. The liver and both kidneys (pooled) were removed while remaining immersed beneath the ice-cold NaCl. Strict emphasis was placed throughout on the rapid chilling and dissecting out of the tissues to ensure the stoppage of any further metabolism beyond the point of killing.

For whole-body measurements of protein synthesis the animals were injected in a manner identical with that described above. After these animals were killed (10min) and exsanguinated, their abdominal and thoracic cavities were opened and the whole animal was immediately frozen in liquid N_2.

Frozen individual tissues, or whole bodies, were pulverized (between chilled metal plates) in a cold-room. Tissue samples were then immediately homogenized in ice-cold 0.3M-$HClO_4$ in a ground-glass homogenizer. Powdered whole bodies were treated similarly after an initial homogenization in 0.3M-$HClO_4$ (1:4, w/v) in a Waring blender. Several (three to five) 10ml samples of the latter preparation were then further homogenized in a ground-glass homogenizer before analysis. The good agreement (i.e. within 8% of the mean) between these three to five samples attested to the homogeneous sampling of the whole-body preparations. The specific radioactivities of the phenylalanine both in the 'flooded' tissue pool(s) and covalently bound in protein were measured in the homogenates of all individual tissues, or whole bodies, by the method described by Garlick *et al.* (1980). This involved the prior hydrolysis of the washed protein pellets in 6M-HCl at 110°C for 24h and the conversion of phenylalanine into β-phen-

ethylamine (Garlick *et al.*, 1980). All measurements of radioactivity were made in a Packard scintillation counter (efficiency for ^3H of 25%) in a Triton X-100/xylene-based scintillant with the use of an external standard. The fractional rate of synthesis (i.e. K_s, the percentage of the protein mass synthesized per day) was calculated from

$$K_s = \frac{S_B}{S_A t} \times 100$$

where S_A and S_B are the specific radioactivities of phenylalanine in the free tissue pool (i.e. intracellular and extracellular) and protein respectively, and t is the time in days.

Proteins were measured on the same tissue or whole-body preparations by the method of Lowry *et al.* (1951) by using bovine serum albumin (Sigma, Kingston upon Thames, Surrey, U.K.) as standard. RNA and DNA were also extracted and measured as previously described (Goldberg & Goldspink, 1975). Growth rates (K_g) were measured as the net accumulation of protein (in either the tissue or whole body, where appropriate) between two time intervals close to, or spanning, the point at which synthesis rates were measured. The exact time intervals varied, being as short as possible in situations of rapid growth (e.g. maximum of 2 days), and becoming longer (i.e. 5–7 days) as growth slowed: precise details are presented in the relevant Tables. Growth rates (K_g) were then expressed as the percentage change in the protein content per day. Since growth arises from an imbalance in the rates of synthesis (K_s) and degradation, protein breakdown (K_b) was calculated by subtraction, from the two measured parameters, i.e. $K_b = K_s - K_g$.

Results and discussion

Developmental changes in the whole animal

Various parameters were initially measured in relation to whole-body growth at, and between, the extremities of the lifespan in this strain of rat (Table 1). From the 14-day foetus to senility, body weight increased approx. 3550-fold, whereas the total protein content increased 9200-fold (Table 1). This disproportionately larger increase in protein reflects both the enlargement of the animal and a continuously increasing protein composition within the tissue mass (i.e. from 4.7 to 13.7%). The overall increase in RNA (1120-fold) was greater than that of DNA (500-fold), as evidenced by the doubling of the RNA/DNA ratios (Table 1). Both nucleic acids, however, were accumulated more slowly than the body mass, as indicated by their decreasing values when expressed per g body wt. For RNA, these declining values were more predominant during post-natal life, changes per g of animal changing little throughout the last 6 days before birth (Table 1). In marked contrast with the rapid growth in the foetus, no significant changes in any of these parameters were recorded between 44 and 105 weeks *post partum*.

To explain the changes in whole-body growth in the foetus and throughout post-natal life, protein synthesis was measured in the whole animal 10min after an injection of phenylalanine. The fractional rate of synthesis (i.e. percentage of the protein mass renewed per day) decreased from 73% in the 14-day foetus to 10.8% in the senile animal (Table 2). Since the rate of protein synthesis depends on the number of ribosomes per cell and the activity of these ribosomes in the translation process, it is

Table 1. *Changes in whole-body weight, protein and nucleic acids during pre- and post-natal life*

Frozen whole bodies were chopped into small pieces by the use of a guillotine and subsequently pulverized between chilled metal plates. The powdered bodies were then allowed to thaw in ice-cold 0.3M-$HClO_4$ (1:4, w/v) before being homogenized in a Waring blender at 3°C. Three to five samples (10ml) of this homogenate were withdrawn and further homogenized in a motor-driven ground-glass homogenizer. Proteins were measured in these fine homogenates by the method of Lowry *et al.* (1951), and RNA and DNA were extracted and measured as described by Goldberg & Goldspink (1975). The values obtained from the three to five separate samples were then averaged to give a single value for each animal. Each value is the mean ± S.E.M. for three to six rats treated as described above.

Age	Body wt. (g)	Total protein (g)	Protein/ wet wt. (%)	Total RNA P (mg)	RNA P/ wet wt. (μg/g)	Total DNA P (mg)	DNA P/ wet wt. (μg/g)	RNA P / DNA P
Foetal (days)								
14	0.17±0.01	0.008±0.001	4.7±0.2	0.06±0.002	364±9	0.06±0.002	379±10	0.96±0.01
16	0.53±0.01	0.027±0.001	4.9±0.4	0.19±0.01	358±13	0.18±0.01	339±9	1.06±0.04
18	1.62±0.02	0.08±0.004	5.1±0.1	0.60±0.01	379±7	0.47±0.01	296±5	1.28±0.02
20	3.61±0.1	0.24±0.008	6.3±0.1	1.32±0.03	351±8	0.98±0.03	264±6	1.32±0.04
Post partum (weeks)								
3	50±0.8	5.6±0.2	11.3±0.3	11.6±0.4	233±7	6.4±0.3	130±5	1.90±0.07
8	211±5	26.4±1.5	12.5±0.7	39.8±1.2	189±8	17.2±0.5	82±3	2.32±0.08
44	584±11	76.8±2.4	13.7±0.6	69.1±1.5	119±3	32.0±1.8	55±4	2.19±0.10
105	604±13	72.6±3.1	12.3±0.7	67.4±2.2	114±6	29.9±1.3	50±3	2.31±0.06

Table 2. *Changes in whole-body protein turnover during pre- and post-natal life*

Protein synthesis was measured in the whole bodies 10min after injection of 150μmol of phenylalanine (containing 65μCi of the ^{3}H-labelled amino acid) per 100g body wt into a lateral tail vein. The exsanguinated bodies were rapidly frozen and subsequently homogenized (see Table 1). The fractional rate of protein synthesis was then calculated after determining the specific radioactivities of both the free and protein-bound phenethylamine in the whole bodies (see the Methods section). The total amount of protein synthesized per day was subsequently calculated for each animal as the product of its fractional rate and protein content (Table 1). This value was then subsequently divided by either the body weight or the RNA content obtained from Table 1. Growth rates were determined as the percentage change in the protein mass of the whole animal per day, protein accumulations being measured over 1-, 2-, 5- or 7-day intervals in the foetuses, 3-, 8-, 44- and 105-week-old rats respectively. The fractional rate of protein breakdown was then calculated by subtracting the growth and synthetic rates.

Age	Fractional rate of synthesis (%/day)	Total protein synthesized (g/day)	Total protein synthesized (g/day per g body wt.)	Total protein synthesized (g/day per g of RNA P)	RNA P/ protein (mg/g)	Growth rate (%/day)	Calculated rate of breakdown (%/day)
Foetal (days)							
14	72.5±2.5	0.006±0.001	33.3±1.9	90±5.2	7.9±0.3	–	–
16	69.4±4.4	0.019±0.002	35.3±2.7	95±6.0	6.9±0.4	63.0	6.4
18	53.1±1.6	0.043±0.002	27.0±1.0	72±3.4	7.5±0.3	46.9	6.2
20	38.4±1.8	0.091±0.006	24.2±1.1	65±3.4	5.5±0.1	–	–
Post partum (weeks)							
3	30.6±0.7	1.72±0.1	34.4±1.4	148±4.0	2.1±0.05	7.3	23.3
8	18.0±1.3	4.68±0.2	22.0±1.2	119±4.0	1.5±0.07	1.88	16.1
44	12.0±0.6	9.59±0.5	18.2±0.9	139±5.2	0.9±0.03	0.23	11.8
105	10.8±1.3	7.86±1.5	13.1±1.4	117±16	0.9±0.03	−0.14	10.9

often useful to examine these parameters in parallel. An index of ribosomal capacity can be obtained from RNA/protein values and ribosomal activity from synthesis per unit of RNA (Henshaw *et al.*, 1971; Waterlow *et al.*, 1978). From such information it is apparent that the decline in the fractional rate of synthesis during foetal life can be attributed to a combined decrease in both the ribosomal capacity and activity (Table 2). After birth, however, the continuing fall in the synthesis rate resulted almost entirely from the progressive decline in the ribosomal capacity, synthesis per ribosome being largely unchanged throughout post-natal life. Although reasonably constant, the average rate of synthesis per ribosome during post-natal growth was significantly higher than that in the foetus just before birth (Table 2). Dietary factors are known to influence ribosomal activity (Henshaw *et al.*, 1971; Rannels *et al.*, 1977; Flaim *et al.*, 1982). However, it is difficult to envisage any major quantitative or qualitative differences in the nutrition of the foetus and weanling rats that might explain these differences (Table 1). At both stages of development these animals were receiving the same diet, albeit via the mother in the case of the foetuses. Indeed, if major dietary changes are manifest anywhere, it might be expected during the period of suckling. An alternative explanation may relate to an increase in the adrenal-cortical activity in the mother shortly before birth. One known action of these steroids is the suppression of peptide initiation (Rannels *et al.*, 1978). Hence before birth the foetal circulation, and therefore its tissues, are probably exposed to this surge of maternal steroids, whereas parturition would bring about the consequent release from their influence (Roudier *et al.*, 1982).

When synthesis rates were calculated to take into account the increasing size of these animals, the total amount of protein being synthesized per day (i.e. $K_s \times$ protein mass) increased approx. 1500-fold (Table 2), the increasing protein content (Table 1) more than compensating for the decreasing fractional rate of synthesis (Table 2). Roughly comparable values of between 2.8 and 10.5g of protein synthesized per day have previously been reported for rats between weaning and 1 year of age (Millward & Lo, 1977; Waterlow *et al.*, 1978; McNurlan & Garlick, 1980). However, differences in animal age (often defined with reference to body weight only), the strain of rat under investigation and the use of different techniques for measuring synthesis preclude a more precise comparison. To our knowledge no rates for the foetus have previously been published. When the same synthesis rates were expressed per unit body weight, the variation narrowed to between 13 and 35g of protein/day per kg (Table 2). These rates were only slightly greater in the foetus and younger rats, and there were no prominent changes which could be readily related to significant events such as birth, weaning or puberty. Similar but wider-ranging (14–80g/day per kg) changes have previously been reported for two other species of rat between wean-

ing and old age (Waterlow *et al.*, 1978; McNurlan & Garlick, 1980). Much less extensive information is so far available for other animal species, including man (Waterlow *et al.*, 1978).

During foetal life the measured growth rates of the whole animal were quantitatively similar to the fractional synthesis rates, thereby suggesting low rates of protein degradation (Table 2). Such a situation would clearly be economical and efficient in enabling rapid growth to occur. This foetal growth appears to arise from both cellular hyperplasia (i.e. a 16-fold increase in DNA; Table 1) and hypertrophy (i.e. a doubling of protein/DNA P, from 0.12 ± 0.01 to 0.24 ± 0.01) between 14 and 20 days *in utero*. In contrast, post-natal growth (beyond weaning) was much slower and primarily attributable to hyperplasia (Table 1), protein/DNA being little changed at 1.6 ± 0.03. These observations *post partum* are in good agreement with the earlier study by Leblond (1972). During the same period of post-natal life the gap between the declining fractional rates of synthesis and breakdown narrowed to establish approximate steady-state conditions, at, and between, 44 and 105 weeks, the half-lives of the mixed proteins being 5.8 and 6.4 days respectively. The higher overall rates of breakdown after birth contrast sharply with the calculated values in the 16- and 18-day old foetuses.

Developmental changes in the liver and kidney

Having defined in detail the developmental changes within the whole animal, we proceeded to investigate the major changes occurring within some of the individual tissues; results for the liver and kidney are presented here, for four different types of muscle in the following paper (Lewis *et al.*, 1984) and for the small and large intestine in the third paper (Goldspink *et al.*, 1984).

Important differences soon became apparent, in the development of the two visceral tissues. Between the 18-day foetus and 105 weeks *post partum*, the liver and kidney increased their weight approx. 125- and 275-fold respectively, compared with a 375-fold increase in body weight. Over the same period, these tissues increased their protein mass some 200- and 525-fold respectively, as a consequence of increasing the protein composition within the expanding tissue mass (Tables 3 and 4). In the kidney the protein content generally increased in line with the changes in the whole animal, both kidneys contributing 0.7–1.9% to the animal's protein mass (Table 4). In marked contrast, the accumulation of protein in the liver fell behind the accretion in the whole animal, such that the hepatic contribution became progressively smaller (from 20 to 4%) with increasing age, the major changes occurring between 18 days in the foetus and weaning (Table 3). The age-related changes in RNA, however, were broadly similar in the whole animal and both visceral tissues, hepatic and renal RNA contributing 16–26% (Table 3) and 1.3–3.0% (Table 4) respectively to the body's RNA (Table 1). Nuclear proliferation (i.e. total DNA) also increased in both developing tissues, but at

Table 3. *Changes in the protein and nucleic acid contents of the liver during pre- and post-natal life*

Livers were reduced to a powder in a manner similar to that described for the whole bodies (Table 1) and homogenized directly (1:50, w/v) in 0.3M-$HClO_4$ in a ground-glass homogenizer. All analytical procedures and measurements were the same as described in Table 1. The values at each age are means ± S.E.M. derived from separate livers from a minimum of nine foetuses or five animals *post partum*. Values in parentheses represent the percentage contribution of the liver towards the whole-body protein or nucleic acid content.

Age	Protein content (mg)	Protein/ wet wt. (%)	Total RNA P (mg)	RNA P/ wet wt. (μg/g)	Total DNA P (mg)	DNA P/ wet wt. (μg/g)	RNA P / DNA P
Foetal (days)							
16	5.4 ± 0.1 (19.6)	9.8 ± 0.5	0.05 ± 0.001 (25.7)	889 ± 48	0.03 ± 0.002 (16.8)	570 ± 36	1.58 ± 0.06
18	16.5 ± 1.1 (20.3)	11.1 ± 0.4	0.16 ± 0.008 (26.8)	1038 ± 24	0.10 ± 0.01 (21.9)	667 ± 40	1.59 ± 0.08
20	30.0 ± 2.4 (12.6)	10.7 ± 0.5	0.27 ± 0.02 (20.4)	974 ± 25	0.15 ± 0.01 (15.4)	526 ± 35	1.90 ± 0.08
Post partum (weeks)							
3	364 ± 11 (6.5)	18.7 ± 0.4	1.82 ± 0.1 (15.8)	871 ± 21	0.44 ± 0.02 (6.9)	211 ± 13	4.18 ± 0.21
8	1860 ± 32 (7.1)	19.1 ± 0.3	7.87 ± 0.2 (18.7)	819 ± 20	1.70 ± 0.03 (9.8)	186 ± 3	4.41 ± 0.06
44	3322 ± 54 (4.3)	20.7 ± 0.4	11.84 ± 0.8 (17.1)	737 ± 25	3.11 ± 0.03 (9.7)	193 ± 19	3.72 ± 0.21
105	3252 ± 19 (4.5)	17.7 ± 0.5	14.1 ± 0.8 (20.9)	800 ± 28	3.32 ± 0.23 (11.1)	189 ± 10	4.29 ± 0.13

Table 4. *Changes in the protein and nucleic acid contents of the kidney during pre- and post-natal life*
Kidneys were analysed in the same manner as the liver (Table 3). Each measurement, however, was made on either pooled kidneys (six) from three foetuses (i.e. a total of 18 tissues) or the left kidney of five or six rats at each age *post partum*. Results for single kidneys are presented as means ± S.E.M. The values in parentheses, however, indicate the percentage contribution that both kidneys make towards the whole body's protein and nucleic acids at each appropriate age. The latter assumes that both kidneys are of equal size throughout development; we have no evidence to the contrary.

Age	Protein content (mg)	Protein/ wet wt. (%)	Total RNA P (μg)	RNA P/ wet wt. (μg/g)	Total DNA P (μg)	DNA P/ wet wt. (μg/g)	RNA P / DNA P
Foetal (days)							
18	0.53±0.05 (1.3)	6.3±0.5	3.8±0.4 (1.3)	488±15	2.7±0.2 (1.2)	343±14	1.38±0.09
20	1.1±0.1 (0.92)	6.9±0.3	9.9±0.6 (1.5)	625±8	6.8±0.5 (1.4)	428±19	1.46±0.05
Post partum (weeks)							
3	55±2.9 (1.9)	16.1±0.3	178±6.3 (3.1)	520±10	109±4.9 (4.9)	300±10	1.61±0.06
8	134±5.1 (1.0)	17.6±0.4	257±12 (1.3)	349±6	183±6 (2.1)	249±14	1.41±0.11
44	271±8.3 (0.71)	17.5±0.4	544±28 (1.6)	352±20	383±17 (2.4)	260±5	1.38±0.05
105	281±8.8 (0.77)	12.2±0.7	690±48 (2.1)	274±29	572±42 (3.8)	237±15	1.24±0.07

rates different from that in the whole animal. The renal contribution to whole-body DNA increased from 1.2 to 4.9%, while leaving the RNA/DNA values largely unchanged at 1.24–1.61 (Table 4). The situation in the liver was very different. Up to weaning, hepatic hyperplasia (i.e. DNA) decreased from 22 to 7% relative to that seen in the whole animal. Thereafter, hepatic DNA remained at close to 10% of that in the whole body (Table 3). Although both nucleic acids were accumulated at similar rates within the foetal liver, between birth and weaning the rate of transcription clearly outstripped nuclear proliferation, this being evident from the doubling of the nucleic acid ratio (Table 3). From weaning onwards, however, the nucleic acid ratio remained reasonably constant (Table 3, and Waterlow *et al.*, 1978), at a higher value than that found in other tissues, e.g. the kidney (Table 4), four different types of muscle and parts of the intestine (Lewis *et al.*, 1984; Goldspink *et al.*, 1984).

In general terms the developmental changes in the kidney paralleled the events in the whole animal. The gradually diminishing contribution of the liver to whole body weight, protein and nucleic acids stand in marked contrast with the changes in the kidney and can perhaps be more readily appreciated by realizing that the 16-day foetus, unlike the adult rat, consists in quantitative terms of little other than a liver, brain and lungs. To investigate further the different patterns of growth within these two visceral tissues, we studied their developmental changes in protein turnover. Before this could be accomplished, however, preliminary experiments were undertaken to establish a suitable time period over which protein synthesis could be measured during both pre- and post-natal life. Precise measurements of synthesis necessitate an accurate knowledge of the specific radioactivities of the tracer amino acid both free in the precursor pool (S_A) and covalently bound in the protein of the tissue (S_B). Ideally S_A should equilibrate rapidly with the plasma, remain constant over the period during which synthesis is measured, and be several times greater than S_B itself (Waterlow *et al.*, 1978). The time-related changes in S_A and S_B after an injection of phenylalanine were therefore determined in the liver and kidney of both 200g rats (Fig. 1*a*) and foetuses at 20 days of gestation (Fig. 1*b*).

In the mature adult animals (i.e. 200g), S_A values for the liver and kidney were already 77 and 94%, respectively, of the specific radioactivity in the plasma at 2.5min (Fig. 1*a*). Values for both tissues and plasma subsequently declined with time, verifying the previous findings of McNurlan *et al.* (1979) and Garlick *et al.* (1980). However, the rate of decline of S_A for phenylalanine was slower than that for leucine (McNurlan *et al.*, 1979). This is not perhaps surprising, since there is proportionately more leucine than phenylalanine in protein, hence protein degradation within the body's tissues would liberate relatively more non-labelled leucine. Between 2.5 and 10min S_A in the liver and

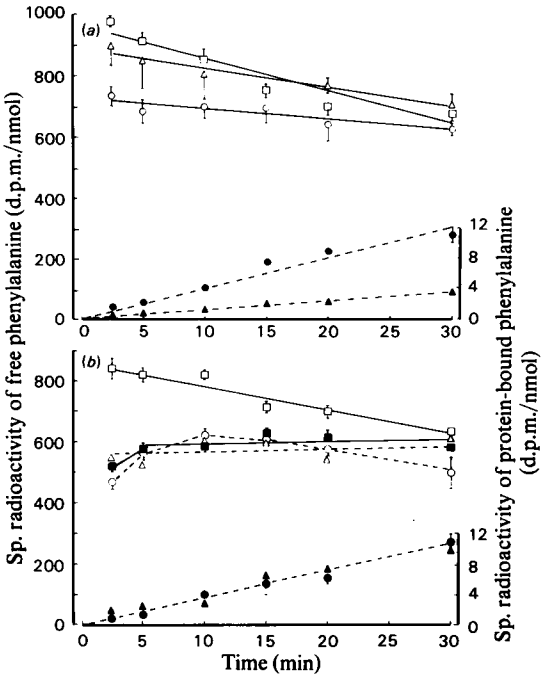

Fig. 1. *Time-related changes in the specific radioactivity of phenylalanine in the plasma, liver and kidney of foetal and adult rats*

(*a*) Rats (200g) were killed at selected times, between 2.5 and 30min, after receiving an intravenous injection containing 150μmol of phenylalanine (including 65μCi of L-[4-^{3}H]phenylalanine) per 100g body wt. The specific radioactivities of the free (i.e. S_A, open symbols) and protein-bound (i.e. S_B, solid symbols) phenylalanine in the plasma (squares), liver (circles) and left kidney (triangles) were determined at each time as described by Garlick *et al.* (1980). Each value is the mean $\pm$S.E.M. for at least four individual tissues. (*b*) Here, 20-day-pregnant rats (270$\pm$10g) were injected in a similar manner to the animals above. The specific radioactivity of the free phenylalanine was then determined in both the maternal (□) and foetal (■) plasma. The free (i.e. S_A, open symbols) and protein-bound (i.e. S_B, solid symbols) specific radioactivities were simultaneously measured in the foetal liver (circles) and kidney (triangles). Values are presented as the means $\pm$S.E.M. Maternal and foetal plasmas were taken from four injected mothers and 16 foetuses. Nine individual livers were analysed, i.e. from three foetuses taken at random from each of the three injected mothers; 18 kidneys from the same foetuses were analysed after pooling six kidneys from each group of three foetuses.

kidney fell by less than 6%. Nonetheless mean S_A values (i.e. at 5.25min) were subsequently taken for measuring the synthetic rates at 10min after injection (Garlick *et al.*, 1980). Again, in agreement with earlier studies (McNurlan *et al.*, 1979; Garlick *et al.*, 1980), S_B within these tissues increased linearly over this time.

Changes in S_A and S_B for the foetal tissues relative to the plasma were also investigated after 20-day-pregnant rats were similarly injected (Fig. 1*b*). The specific radioactivity of phenylalanine in the foetal plasma was found to have equilibrated with the maternal plasma at the earliest time studied (i.e. 2.5min), indicating very rapid transfer of the amino acid across the placenta. Throughout the 30min studied, the specific radioactivity in the foetal blood was between 60 and 95% of that in the maternal plasma. However, although the free specific radioactivity fell slowly with time in the maternal circulation (Fig. 1*a*), it remained constant in the foetal blood (Fig. 1*b*). This interesting difference between the two circulations probably relates to the very different rates of protein breakdown in the foetal and adult tissues (see below), thereby releasing different quantities of non-labelled phenylalanine into the appropriate circulations. Equilibration between the foetal plasma and these two tissues was equally rapid and essentially complete 2.5min after the injection (Fig. 1*b*). Although it remained constant thereafter in the kidney, there was a suggestion that S_A reached a peak slightly later (i.e. after 5min) in the foetal liver. Although we have no good reason to doubt this value at 5min, it may be slightly misleading in that it is some 10% higher than that measured in the plasma at the same time. We have therefore assumed that S_A in the liver is constant between 2.5 and 30min, as is the case for the kidney (Fig. 1*b*) and other tissues [see the following two papers (Lewis *et al.*, 1984; Goldspink *et al.*, 1984)]. This assumption for the liver appears to be validated by the narrow range (from 88.1$\pm$5.0% to 104$\pm$5.4%) of fractional rates of synthesis when calcuated from each of the time points studied.

Problems associated with identifying the precise precursor pool(s) for synthesizing new proteins are largely overcome by injecting such large doses of the tracer amino acid, the tissue pool (S_A) being expanded so that its specific radioactivity is close to that of the plasma. This is particularly so in foetal tissues, where S_A was found to be 91–104% of that in the foetal plasma (Fig. 1*b*). The injection of this large dose of the amino acid does not itself alter the rate of protein synthesis (McNurlan *et al.*, 1979). The incorporation of phenylalanine into protein (S_B) was linear (Fig. 1*b*) in both foetal tissues over the time studied, and in all cases S_B was less than 2% of S_A. Since S_A remained constant, synthesis (and S_A) was subsequently only measured in the foetal tissues 10min after injection of phenylalanine. It was clear from these initial studies that accurate rates of protein synthesis could be

measured in both tissues during pre- and post-natal growth after a 10min injection of the radioactive tracer.

As in the whole animal, growth of the liver and kidney slowed dramatically during the last few days before birth and up to weaning (see growth rates, Tables 5 and 6). Before weaning, tissue growth had occurred as a combination of hyperplasia and hypertrophy (Tables 3–6). However, since cell size (as defined by protein/DNA values)

Table 5. *Changes in protein turnover in the liver during pre- and post-natal growth*

The values below are the means ± S.E.M. of measurements made on the same liver preparations as used in Table 3. All analytical procedures were the same as described in Table 2. The values in parentheses represent the percentage contribution that hepatic protein synthesis makes towards total synthesis in the whole animal (i.e. Table 2). Growth rates were measured in the livers over the same time intervals as described in Table 2. The rate of breakdown at each age was subsequently calculated by subtraction of the growth rate from the fractional synthetic rates × 0.7. The latter correction allows for the proportion of hepatic protein synthesized that is subsequently secreted (see the text, and Scornik & Botbol, 1976).

Age	Protein/DNA (mg/μg)	Fractional rate of synthesis (%/day)	Total protein synthesized (mg/day)	Total protein synthesized (g/day per g of RNA P)	RNA P/protein (mg/g)	Growth rate (%/day)	Calculated rate of breakdown (%/day)
Foetal (days)							
16	0.17±0.01	112±10	6.0±0.51 (32.4)	123±10	9.1±0.2	51.4	27.0
18	0.17±0.1	134±5.9	24.1±2.6 (56.2)	146±10	9.4±0.3	35.9	57.9
20	0.21±0.02	97.5±5.5	27.3±2.7 (29.9)	95.0±6	8.8±0.4	20.9	47.4
Post partum (weeks)							
3	0.81±0.1	52.5±1.5	187±6 (10.9)	105±1	4.8±0.4	8.5	28.3
8	1.20±0.1	58.8±1.7	1189±20 (25.4)	149±4	4.2±0.2	4.9	36.3
44	1.07±0.1	42.5±2.0	1477±34 (15.4)	132±10	3.6±0.1	1.3	28.5
105	0.95±0.03	48.0±4.1	1588±19 (20.2)	119±10	4.5±0.1	0.43	33.2

Table 6. *Changes in protein turnover in the kidney during pre- and post-natal life*

Rates of protein synthesis were measured in the same kidneys as described in Table 4. The percentage contributions that both kidneys make towards whole-body protein synthesis are presented in parentheses. Growth rates in the left kidney were measured as percentages of the protein mass accumulated over the time intervals described in Table 2. Subtraction of this value from the fractional synthetic rate yields the rate of breakdown at each age.

Age	Protein/DNA (mg/μg)	Fractional rate of synthesis (%/day)	Total protein synthesized (mg/day)	Total protein synthesized (g/day per g of RNA P)	RNA P/protein (mg/g)	Growth rate (%/day)	Calculated rate of breakdown (%/day)
Foetal (days)							
18	0.20±0.01	94.9±6.7	0.51±0.1 (2.4)	122±16	7.2±0.4	54.3	40.6
20	0.17±0.01	62.7±9.8	0.68±0.1 (1.5)	69.5±12	9.1±0.4	26.2	36.6
Post partum (weeks)							
3	0.57±0.04	44.7±2.3	24.6±3.3 (2.9)	147±6.0	3.3±0.1	2.94	41.8
8	0.78±0.05	31.8±1.9	40.9±1.6 (1.8)	161±6.5	1.9±0.1	1.85	30.0
44	0.76±0.01	30.8±2.1	76.0±3.9 (1.6)	157±8.1	2.0±0.1	1.55	29.2
105	0.53±0.04	23.5±0.9	69.7±6.0 (1.8)	107±5.0	2.2±0.2	0.03	23.2

appeared to be largely unchanged after weaning, the continued growth of both tissues (Tables 5 and 6) through to senility primarily arises from hyperplasia (see also Leblond, 1972). Although protein/DNA is a useful index of cell size for cells possessing a single diploid nucleus, the liver does exhibit some polyploidy, and its incidence may vary with different stages of development (Van Bezooijen *et al.*, 1972). Hence there may be some overestimate of cell number in this particular tissue. Nonetheless, it is unlikely that this alone could account for the large pre- and post-natal changes in the protein/DNA values (Table 5). Throughout development the fractional rates of protein synthesis and breakdown were consistently higher in the liver (Table 5) than in the kidney (Table 6), which in turn were higher than those in whole animal (Table 2). The greater overall decline in these rates within the whole body (Table 2) must be explained by the more marked post-natal changes occurring in other tissues, which also possess lower absolute rates, e.g. the musculature (Lewis *et al.*, 1984). Although synthesis per ribosome, like the fractional rate, was generally higher in the visceral tissues compared with the whole foetus (Tables 2, 5 and 6), these particular differences disappeared during post-natal life.

Other significant developmental differences between these two visceral tissues were also apparent. Although decreasing during foetal life, the fractional rate of synthesis in the liver was relatively unchanged between weaning and senility, thus correlating with a fairly stable ribosomal capacity and activity *post partum*. In contrast, renal synthesis continued to decline right through to senility, the rate at 105 weeks being approximately one-half of that measured at 3 weeks. This difference correlated with a progressive decrease in the number of renal ribosomes. Synthesis per ribosome in the kidney was, however, broadly similar through both intrauterine and post-natal life, except for two interesting stages of development (Table 6), i.e. 20 days of gestation and 105 weeks *post partum*. Similar, but less clear-cut, changes were also evident in the liver at these specific times (Table 5). The suppression of protein synthesis per unit of RNA in both foetal tissues at 20 days was similar to that observed in the whole animal (Table 2) immediately before birth, and may (as suggested above) result from the transient surge in maternal steroids. In contrast, the decline in this rate and in the absolute amount of protein synthesized per day in the senescent animal (Table 2), its kidneys (Table 6) and musculature (see Lewis *et al.*, 1984) may represent the beginnings of a reversal of growth, i.e. the early stages of old-age atrophy.

Throughout, the total amount of renal protein being synthesized per day remained reasonably constant, at between 1.5 and 2.9% of that in the animal as a whole. Although much more variable, the hepatic contribution to whole-body synthesis appeared to fall with increasing age (ranging from 56 to 11%; Table 5). Our measurements of the fractional and total rates of synthesis for the liver and kidney *post partum* are generally in good agreement with rates published previously for the rat (Waterlow *et al.*, 1978), despite differences in methodology. The same rates do, however, tend to be slightly lower than those published by other workers using the same method (McNurlan *et al.*, 1979; McNurlan & Garlick, 1980; Garlick *et al.*, 1980). Such differences must presumably relate to the use of different strains of rat raised on different diets. If so, this further emphasizes the need to control as many variables as possible within any investigation of an age-related nature.

Except for the whole foetus, the rates of protein breakdown in the whole animal (Table 2) and both visceral tissues (Tables 5 and 6) paralleled the changes in the fractional synthetic rates, the slow declining rate of breakdown in the kidney being intermediate with respect to the rapid decline in the whole body rate and the fairly constant hepatic rate *post partum*. Direct measurements of protein degradation *in vivo* are often subject to error because of the high degree of reutilization of most tracer amino acids and the non-exponential decay of label in mixed proteins. As an alternative, breakdown may be calculated, as here, by subtracting the measured rates of growth and synthesis. The advantages and short-comings of this indirect method of determining breakdown have been discussed in detail elsewhere (Garlick *et al.*, 1979). The age-related rates of breakdown in the kidney and liver were broadly similar after allowance was made for the loss of plasma proteins from the livers (Table 5). The latter correction was necessary because, over the short period during which synthesis was measured, all hepatic proteins are measured, including those destined for export. The subsequent loss of these plasma proteins (approx. 30% of hepatic protein; Scornik & Botbol, 1976) would not, however, be included within the protein masses and hence the growth rates (Table 5), when measured over the longer intervals of 1 or more days. In the absence of information about the loss of plasma proteins from foetal livers, the same corrective procedure was applied throughout, regardless of age (Table 5).

It is difficult to see how errors in the measurements of either synthesis or growth could alone account for the low calculated rates of breakdown in the whole foetus (Table 2), since such errors should be equally applicable to the measurements within the individual tissues (Tables 5 and 6). However, if the foetal whole-body rates (i.e. as a

tissue average) are accurate, this means that some foetal tissues must exhibit very low rates of degradation before birth to offset the much higher rates in the visceral tissues (Tables 5 and 6). The half-life values for mixed liver and kidney proteins, based on the synthesis rates measured under near-steady-state conditions (i.e. 105 weeks), were found to be 1.45 and 2.95 days respectively, compared with 6.4 days for the whole animal.

Clearly different patterns of growth and changes in protein turnover are to be found within individual tissues of the body, and this is discussed in more detail by Goldspink *et al.* (1984).

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