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# Protein synthesis during the developmental growth of the small and large intestine of the rat

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1. The developmental growth and associated changes in protein synthesis were measured (*in vivo*) in the combined small and large intestine from 18 days *in utero* to 105 weeks *post partum*. Similar post-natal (3–105 weeks) changes were also studied in the separated large and small intestine, and in the mucosal and muscularis externa + serosal layers of the small intestine. 2. Although the protein and nucleic acid contents of the whole intestine increased throughout both pre- and post-natal life, the maximal (11%) intestinal contribution to whole-body growth occurred 3 weeks after birth; this value declined to only 2.5–3.5% at both extremes of the age range studied. Between the 18-day foetus and old age the fractional rate of protein synthesis decreased from 107 to 61% per day. This developmental decline (43%) was, however, much smaller than that found in most other body tissues over the same period. 3. Similar developmental trends (between weaning and senility) were found in both the small and the large intestine when studied separately, the small intestine in all respects contributing proportionately more than the large intestine to both the combined intestinal and whole-body values. At each age the large intestine possessed significantly lower fractional rates of synthesis and associated ribosomal activities. For the most part, the fractional synthesis rates in the mucosa and serosa of the small intestine were very similar, with each declining slightly with increasing age. These developmental changes are discussed with respect to functional aspects within the gastrointestinal tract.

Several studies have described very rapid turnover rates in various parts of the gastrointestinal tract (McNurlan *et al.*, 1979; McNurlan & Garlick, 1980; Wassner & Li, 1982; Kelly & Goldspink, 1982). Hence the gut as a whole makes disproportionately larger contributions to the body's rate of protein synthesis than to its protein mass. However, a necessary prerequisite for measuring meaningful rates of synthesis in the gut, as elsewhere in the body, is the availability of a reliable technique, one equally applicable to the tissue in question and to the whole animal. Major technical difficulties usually involve identifying the precursor amino acid pool at the site of protein synthesis and measuring the rapidly changing specific radioactivities of tracer amino acids when introduced into that pool (Waterlow *et al.*, 1978). These problems have been particularly apparent with studies involving the gastrointestinal tract, since precursor amino acids could conceivably be drawn from intraluminal, as well as tissue and plasma, sources (Fern *et al.*, 1971; Alpers, 1972; Alpers & Kinzie, 1973; Fern & Garlick, 1974). The modification (Garlick *et al.*, 1980) of the technique of Henshaw *et al.* (1971) largely overcomes such problems. The injection of a large dose of an amino acid effectively floods the precursor pool(s), allowing synthesis rates to be measured over a short period of time during which the specific radioactivity of the free amino acid remains relatively constant.

To our knowledge no previous study has defined the developmental changes in the growth and protein turnover of the large and small intestine in relation to changes in the whole animal. Throughout, from the 18-day foetus to senility (105 weeks), we have utilized the same large-dose method

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(Garlick *et al.*, 1980) for measuring synthesis rates in both the whole animal and in each part of the intestine.

## Methods

Fuller details of the animals and techniques used are described by Goldspink & Kelly (1984) and Garlick *et al.* (1980).

Animals *post partum* were decapitated either 2.5 or 10min after receiving an intravenous injection of the large dose of phenylalanine. The abdominal cavity of each rat was immediately opened and the entire animal submerged in ice-cold 0.9% NaCl to chill the gastrointestinal tract rapidly. Where pregnant rats were used to obtain foetal tissues, three foetuses were chosen at random and immediately removed from the chilled uterus. The abdominal cavity of each foetus was in turn opened to allow rapid cooling of its gastrointestinal tract. The entire intestine, from duodenum to anus, was isolated from the foetuses, and weighed and frozen in liquid $N_2$. In the larger rats (i.e. *post partum*) the entire small (i.e. duodenum plus jejunum and ileum) and large (caecum to anus) intestines were isolated and flushed with ice-cold 0.9% NaCl to remove all dietary and faecal contents. The large intestine was opened along its length, blotted to remove excess moisture and then frozen in liquid $N_2$. To ensure adequate chilling throughout, the small intestine was cut into sections, each being briefly removed from the saline to be treated accordingly. Each section in turn was slit along its length, and the mucosal layer was removed by scraping the tissue with the edge of a microscope slide (McNurlan *et al.*, 1979). The remaining muscularis externa + serosa was then gently wiped with a paper tissue to remove any contaminating mucosal cells. The separated mucosal and serosal layers were then frozen in liquid $N_2$. Before analysis the frozen tissues were pulverized and samples homogenized in ice-cold 0.3M-$HClO_4$ (1:50, w/v). Nucleic acids were extracted and measured as described previously (Goldberg & Goldspink, 1975) and proteins were determined by the method of Lowry *et al.* (1951). The specific radioactivities of phenylalanine in the tissue pool (i.e. $S_A$) and covalently bound in protein ($S_B$) were measured by the method of Garlick *et al.* (1980), and the fractional rate of protein synthesis ($K_s$), (%/day) was calculated from the equation

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

where $t$ is the time in days.

## Results and discussion

Before studying the developmental growth and associated changes in protein turnover within the intestine, it was necessary to establish once again the suitability of the large-dose method (McNurlan *et al.*, 1979; Garlick *et al.*, 1980) for measuring synthetic rates in the gastrointestinal tract, both before and after birth. These preliminary experiments were undertaken in a manner identical with those described in the two preceding papers (Goldspink & Kelly, 1984; Lewis *et al.*, 1984). The free and protein-bound specific radioactivities of phenylalanine were measured in the mucosal and serosal layers of the small intestine of 200g adult rats (Fig. 1*a*) and in the combined small and large intestine of 20-day foetuses (Fig. 1*b*). The previous findings of others (McNurlan *et al.*, 1979; Garlick *et al.*, 1980) were largely confirmed in the studies on adult animals. The free specific radioactivity of the amino acid in the mucosa and serosa rapidly equilibrated with that of the plasma, being respectively 70 and 78% that of the plasma at 2.5min. The subsequent linear decline in all of these values with time relates to the rapid turnover of the intestinal proteins. This contrasts with the much slower turnover and hence constancy of the free pool in striated muscles over the same time (Lewis *et al.*, 1984). Although the free specific radioactivity in the mucosa and serosa fell by less than 8% over the 10min after commencing the intravenous injection, a mean value (i.e. at 5min, derived from animals killed at both 2.5 and 10min; McNurlan *et al.*, 1979) was used. Protein-bound specific radioactivities, however, were only measured after 10min, since the incorporation of phenylalanine into protein was clearly linear for at least 30min after its injection (Fig. 1*a*).

The administration of the large dose of phenylalanine via the mother's circulation gave rise to very similar events to those described in the liver, kidney and muscles of the 20-day foetus (Goldspink & Kelly, 1984; Lewis *et al.*, 1984). In this case the whole intestine was analysed, it not being possible to separate meaningfully the large and small intestine, nor the mucosa from the serosa in the latter. The injected phenylalanine clearly rapidly equilibrated between the maternal and foetal circulations and the foetal intestine (Fig. 1*b*). Over the 30min investigated the free specific radioactivity remained unchanged while the amino acid was linearly incorporated into protein. Subsequent measurements of both specific radioactivities were only made 10min after injection. This large-dose method used over 10min appears to be appropriate for obtaining precise rates of synthesis in both foetal and adult tissues. In the latter, and in particular in tissues where turnover rates are

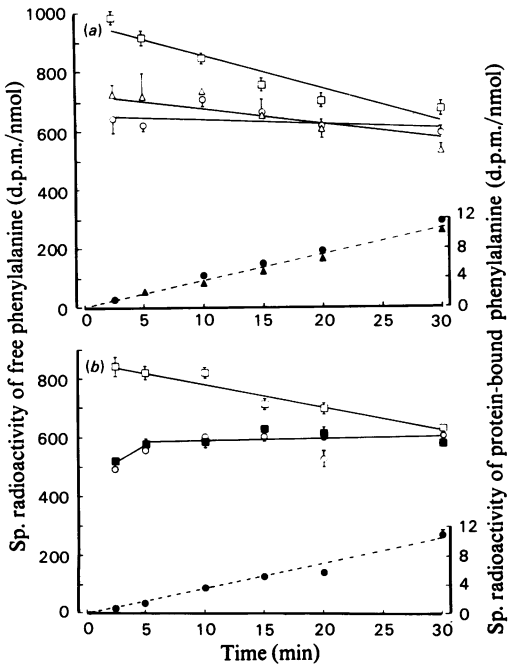


Fig. 1. *Changes in the specific radioactivity of phenylalanine in the plasma and intestine of foetal and adult rats*
(*a*) Adult rats (200g) were killed at selected times after receiving an intravenous injection of phenylalanine (150$\mu$mol, including 65$\mu$Ci of L-[4-$^3$H]phenylalanine per 100g body wt.). At each time 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), mucosa (circles) and muscularis externa + serosa (triangles) were determined, as described by Garlick *et al.* (1980). Each value is the mean ± S.E.M. for at least four individual tissue preparations. (*b*) Here 20-day pregnant rats (270 ± 10g) were injected similarly. The specific radioactivity of the free phenylalanine in both the maternal (□) and foetal (■) plasma was determined at each time, plasma samples being taken from four injected mothers and 16 foetuses. The free (i.e. $S_A$, open symbols) and protein-bound (i.e. $S_B$, solid symbols) specific radioactivities in the entire small and large intestine (circles) of nine foetuses was simultaneously measured; values are presented as the means ± S.E.M.

high, mean free-specific-radioactivity measurements (e.g. at 5min) as defined by McNurlan *et al.* (1979) may be necessary. The rapid flooding of all possible precursor pools so that they reach nearly the same specific radioactivity overcomes many of the previous problems encountered in studying protein synthesis within the gastrointestinal tract (Fern *et al.*, 1971; Alpers, 1972).

*Developmental changes in the small and large intestine*

The age-related changes in intestinal growth from the foetus to senility could only be studied by combining the small and large intestine (Tables 1 and 2). The larger tissues *post partum* did, however, enable a more detailed investigation of the relative changes in the small and large intestine, and between the mucosal and serosal layers of the former (Table 3).

From the 18-day foetus to old age the protein and nucleic acid contents of the combined intestine progressively increased, throughout post-natal life the small intestine making substantially greater contributions to this end (Table 1). Although very large increases in all of these parameters occurred over the period of suckling, the overall rate of growth was clearly slowing over the developmental period studied (i.e. from approx. 70 to 0.05% per day). However, growth of the small and large intestine (either separately or combined) clearly outstripped growth in the whole animal (Goldspink & Kelly, 1984), the intestinal contributions to the body's protein mass being maximal at weaning (i.e. 3 weeks). This probably represents the physiological adaptation to the sudden utilization of the gut immediately after birth. This can presumably be envisaged as increased work demands imposed on the circular and longitudinal sheets of smooth muscle in the large and small intestine and the developmental growth of the numerous absorptive villi in response to the appearance of products of digestion.

In contrast with most other body tissues (Goldspink & Kelly, 1984; Lewis *et al.*, 1984), the protein content of all parts of the intestine was low (maximum 16% of wet wt.) and changed little during normal development and in old age itself (Table 1). Similarly, the nucleic acid ratios were essentially unchanged during both pre- and post-natal life, the values in the small intestine being consistently higher than those measured in the large intestine (Table 1). Growth in the intestine must primarily arise as hyperplasia, with a 445-fold increase in DNA between the 18-day foetus and 105 weeks *post partum*. An approximate doubling of cell size (protein/DNA) was also evident, but this was limited to a short period between birth and weaning (Table 2). These findings are broadly in agreement with a description of the nature of the post-natal growth (7–90 days) in the entire digestive tract (Leblond, 1972). Although such growth in the gastrointestinal tract appears to be mainly accomplished by hyperplasia, the actual number of cells added each day has been estimated to be only 1% in 240g rats (Leblond, 1964). This means that the high rates of mitosis to produce new columnar

Table 1. *Pre- and post-natal changes in the protein and nucleic acid contents of the small and large intestine*
The frozen small (SI) and large (LI) intestines, either combined (i.e. SI + LI) or individually, were first pulverized into a fine powder. A sample of this was subsequently homogenized in ice-cold 0.3M-$HClO_4$ (1:50, w/v) in a motor-driven ground-glass homogenizer. Protein was then measured by the method of Lowry *et al.* (1951), and nucleic acids were extracted and assayed as described previously (Goldberg & Goldspink, 1975). Each value is the mean ± S.E.M. for five to eight foetal or adult tissues. Values in parentheses or brackets show the percentage contribution of either the combined small and large intestine (parentheses) or each separately (square brackets) with respect to whole-body values as described in Goldspink & Kelly (1984).

| 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) | | | | | | | | |
| 18 | SI + LI | 2.5 ± 0.1 (3.1) | 7.8 ± 0.3 | 0.021 ± 0.002 (3.5) | 599 ± 17 | 0.013 ± 0.001 (2.7) | 356 ± 32 | 1.77 ± 1.4 |
| 20 | SI + LI | 8.4 ± 0.6 (3.5) | 9.8 ± 0.6 | 0.06 ± 0.003 (4.6) | 693 ± 23 | 0.04 ± 0.002 (4.0) | 451 ± 11 | 1.54 ± 0.1 |
| *Post partum* (weeks) | | | | | | | | |
| 3 | SI + LI | 606 ± 34 (10.8) | 14.2 ± 0.6 | 2.41 ± 0.1 (20.8) | 568 ± 21 | 1.56 ± 0.1 (24.3) | 356 ± 22 | 1.65 ± 0.1 |
| | SI | 548 ± 29 [9.8] | 14.0 ± 0.5 | 2.24 ± 0.1 [19.4] | 582 ± 21 | 1.39 ± 0.07 [21.6] | 363 ± 16 | 1.54 ± 0.03 |
| | LI | 65 ± 4.5 [1.2] | 16.1 ± 0.8 | 0.19 ± 0.03 [1.6] | 452 ± 37 | 0.15 ± 0.02 [2.3] | 368 ± 17 | 1.30 ± 0.1 |
| 8 | SI + LI | 1555 ± 182 (5.9) | 12.5 ± 1.2 | 6.06 ± 0.6 (15.2) | 486 ± 34 | 3.73 ± 0.4 (21.7) | 299 ± 26 | 1.71 ± 0.1 |
| | SI | 1271 ± 169 [4.8] | 12.0 ± 1.4 | 5.37 ± 0.6 [13.5] | 503 ± 42 | 3.20 ± 0.4 [18.6] | 299 ± 31 | 1.70 ± 0.1 |
| | LI | 289 ± 13 [1.1] | 15.7 ± 0.5 | 0.69 ± 0.7 [1.7] | 382 ± 28 | 0.53 ± 0.04 [3.0] | 293 ± 14 | 1.35 ± 0.06 |
| 105 | SI + LI | 1893 ± 83 [2.6] | 10.3 ± 0.6 | 10.6 ± 0.9 (15.7) | 571 ± 40 | 5.81 ± 0.6 (19.4) | 334 ± 16 | 1.87 ± 0.1 |
| | SI | 1504 ± 50 [2.1] | 9.7 ± 0.7 | 9.39 ± 0.7 [13.9] | 600 ± 41 | 4.81 ± 0.6 [16.1] | 331 ± 28 | 2.02 ± 0.2 |
| | LI | 349 ± 31 [0.48] | 13.1 ± 1.1 | 0.91 ± 0.1 [1.3] | 355 ± 36 | 1.0 ± 0.1 [3.3] | 346 ± 7 | 1.18 ± 0.2 |

epithelial cells only slightly exceeds the rate at which whole epithelial cells are shed (approx. 30% of the population per day) from the extrusion zone into the lumen. Although this daily production of new cells increases as the cell population and tissue enlarges, a steady state is reached around 12–15 weeks *post partum*, when cell production and cell loss are balanced (Leblond, 1972). In part, this steady state is achieved by an increase in the rate of cell migration from the crypt areas to the tips of the villi and an increase in their subsequent extrusion (Koldovsky *et al.*, 1966). Although those earlier studies were not solely confined to the intestine, they are clearly very relevant to the mobile and highly dynamic epithelial cells found in the mucosa (see below).

These changes in cell number and size within the developing intestine were accompanied by a progressive decline in the fractional rate of protein synthesis (Table 2). The overall fall of 43% in the entire intestine between 18 days *in utero* and old age is much smaller than that found in the musculature, liver or kidney over the same period of development (Goldspink & Kelly, 1984; Lewis *et al.*, 1984). However, unlike most other body tissues, the intestine maintains its high rate of DNA and protein synthesis during post-natal life in association with this continuous proliferation, differentiation and extrusion of columnar epithelial cells. It is noteworthy that the maximum percentage contribution of the intestine to whole-body synthesis, like that of growth (see above), occurred at 3 weeks, progressively diminishing thereafter (Table 2). Age for age, both the fractional and total rates of synthesis were always significantly higher in the small intestine, a finding which is in good agreement with the rates published by others (McNurlan & Garlick, 1980). The post-natal decrease in the fractional synthesis rate was slightly more pronounced in the large intestine than in the small intestine (i.e. 44% compared with 33%). However, the greater protein mass and higher rates in the

Table 2. *Pre- and post-natal changes in protein synthesis in the small and large intestine*
Protein synthesis was simultaneously measured in the tissues used for Table 1, i.e. in either the combined small and large intestines (i.e. SI + LI) or in each separately, 10 min after intravenous injection of 150 μmol of phenylalanine/100 g body wt. The fractional rate of synthesis was then calculated after determining the specific radioactivities of both the free (i.e. mean value at 5 min) and protein-bound (at 10 min) phenethylamine in the intestinal preparations. The amount of protein synthesized per day was subsequently calculated as the product of the fractional rate and protein mass. Values in parentheses or brackets show the percentage contribution of either the entire small and large intestine (parentheses) or each separately (square brackets) with respect to whole-body rates of synthesis (Goldspink & Kelly, 1984).

| Age | | Protein/DNA P (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) |
|---|---|---|---|---|---|---|
| Foetal (days) | | | | | | |
| 18 | SI+LI | 0.23±0.02 | 107.0±8.4 | 2.9±0.3 (6.7) | 137±7.6 | 7.70±0.2 |
| 20 | SI+LI | 0.22±0.02 | 85.6±5.8 | 8.8±0.9 (9.7) | 137±10 | 6.92±0.4 |
| *Post partum* (weeks) | | | | | | |
| 3 | SI+LI | 0.42±0.02 | 93.6±2.9 | 567±38 (33.0) | 235±12 | 4.02±0.2 |
| | SI | 0.39±0.02 | 97.5±3.1 | 535±36 [31.1] | 239±14 | 4.13±0.2 |
| | LI | 0.44±0.03 | 57.8±4.3 | 34.9±2.8 [2.0] | 198±12 | 2.94±0.3 |
| 8 | SI+LI | 0.42±0.03 | 78.3±6.9 | 1197±23.8 (25.6) | 193±24 | 3.97±0.3 |
| | SI | 0.44±0.02 | 82.4±8.8 | 1082±23 [23.1] | 196±26 | 3.94±0.1 |
| | LI | 0.51±0.05 | 41.1±1.9 | 127±7.0 [2.7] | 175±14 | 2.44±0.2 |
| 105 | SI+LI | 0.34±0.02 | 60.8±3.3 | 1157±99 (14.6) | 110±5.7 | 5.57±0.3 |
| | SI | 0.33±0.03 | 64.4±3.7 | 975±86 [12.6] | 105±8.8 | 6.23±0.4 |
| | LI | 0.38±0.03 | 32.4±4.5 | 120±36 [1.6] | 118±22 | 3.11±0.3 |

small intestine invariably mean that the small intestine exerts a greater influence over the post-natal trends in the combined intestinal preparation (Tables 1 and 2). The age-related decline in the fractional rate in the small and large intestine (individually or collectively) correlated with successive decreases in the ribosomal capacities (i.e. RNA/protein) up to 8 weeks *post partum*. Interestingly, the rate of synthesis per ribosome increased significantly after birth (Table 2) to a value in excess of that found in most other body tissues (Goldspink & Kelly, 1984; Lewis *et al.*, 1984). Up to 8 weeks after birth, synthesis per unit of RNA in the large intestine was consistently lower than that found in the small intestine.

In the intestines of the senescent animals there was a suggestion of cellular atrophy (i.e. decreased protein/DNA values; Table 2) and small decreases in both the percentage protein composition (Table 1) and the total amount of protein being synthesized per day (Table 2), compared with values measured at 8 weeks. These changes were, however, accompanied by curious antagonistic changes (Table 2) in the ribosomal capacities (i.e. increased) and ribosomal activities (i.e. decreased). Unfortunately growth rates could not be measured in the small number of senile animals available, hence it was not possible to indicate how these changes within the gut might possibly relate to old-age atrophy, as described for other tissues (Goldspink & Kelly, 1984; Lewis *et al.*, 1984). Certainly aging is accompanied by a decrease in gut motility (Rockstein, 1975), and this could result in atrophy of the smooth-muscle components of the large and small intestine. A decrease in the number of intestinal villi has also been noted in old rats

Table 3. *Post-natal changes in protein synthesis in the mucosa and muscularis externa+serosa of the small intestine*
The mucosa and muscularis externa + serosa of the entire small intestine were initially separated (see the Methods section). These were then analysed separately with respect to their protein and nucleic acid contents and protein-synthesis rates by using the same procedures as described in Tables 1 and 2. A minimum of five small intestines were used at each age, with values presented as means ± S.E.M. The percentage contributions of the mucosa and serosa to whole-body values are given in parentheses.

| Age | | Protein content (mg) | Total RNA P (mg) | Total DNA P (mg) | Protein synthesis (%/day) | Protein synthesis (mg/day) | Protein synthesis (g/day per g of RNA P) | RNA P/protein (mg/g) |
|---|---|---|---|---|---|---|---|---|
| *Post partum* (weeks) | | | | | | | | |
| 3 | Mucosa | 157 ± 9 (2.81) | 0.72 ± 0.4 (6.23) | 0.35 ± 0.02 (5.47) | 90.2 ± 5.1 | 141 ± 12 (8.20) | 194 ± 2.5 | 4.66 ± 0.1 |
| | Serosa | 392 ± 27 (7.01) | 1.52 ± 0.9 (13.1) | 1.04 ± 0.06 (16.2) | 100.1 ± 3.9 | 394 ± 35 (22.9) | 259 ± 18 | 3.93 ± 0.2 |
| 8 | Mucosa | 341 ± 46 (1.29) | 1.65 ± 0.2 (4.15) | 0.54 ± 0.4 (3.15) | 87.7 ± 9.1 | 299 ± 65 (6.39) | 171 ± 13 | 4.81 ± 0.2 |
| | Serosa | 1039 ± 109 (3.93) | 3.71 ± 0.5 (9.33) | 2.61 ± 0.3 (15.2) | 92.6 ± 6.8 | 998 ± 22 (21.3) | 265 ± 8.7 | 3.59 ± 0.3 |
| 105 | Mucosa | 561 ± 43 (0.77) | 4.39 ± 0.3 (6.51) | 1.77 ± 0.1 (5.92) | 80.8 ± 3.0 | 457 ± 51 (5.81) | 104 ± 6.3 | 7.90 ± 0.4 |
| | Serosa | 943 ± 48 (1.30) | 4.99 ± 0.5 (7.40) | 3.41 ± 0.6 (11.4) | 54.5 ± 4.1 | 518 ± 53 (6.59) | 95 ± 11 | 5.34 ± 0.6 |

(Clarke, 1972). Further work will clearly be needed to clarify the true significance of the changes in the gastrointestinal tract of the senile animals.

*Post-natal changes in the mucosa and muscularis externa + serosa of the small intestine*

Although most of the developmental changes were predictably very similar to those described above for the entire small intestine, some differences are worthy of mention (Table 3). Without exception, at each age the muscularis externa + serosa made larger contributions to the protein and nucleic acid masses of both the small intestine and whole body (Table 3). However, the respective contributions towards whole-body growth (i.e. protein) decreased with age; this was true of both the serosa (from 7.0 to 1.3%) and mucosa (from 2.8 to 0.8%). The protein composition within each preparation also decreased with age, but always remained higher in the serosal layer (i.e. 16.0–10.5%, compared with 11.2–8.6%). In contrast, the nucleic acid ratios were consistently and significantly higher in the mucosa (2.1–2.5, compared with 1.45–1.6), and remained unchanged from weaning onwards. In endeavouring to explain the growth of the mucosa and serosa, we were surprised to find very similar fractional rates of synthesis in both tissue preparations (Table 3). The few previous studies have described very different synthesis rates for the mucosa and serosa, the rate of the former in the jejunum being approximately double that of the latter (Garlick *et al.*, 1980; McNurlan & Garlick, 1981). Only in the senile rats of our study was the serosal fractional rate significantly lower (33%) than that measured in the mucosa (Table 3). We can only offer two possible explanations for the apparent differences in the results of the two research teams concerned. Firstly there is the possibility of some cross-contamination between the two preparations. To counter this possibility, the same individual in our laboratory always performed the isolation procedure to ensure reproducibility at each age within our study. Hence we believe that the differences at 105 weeks represent a preferential effect of aging on the serosa, probably arising from a decrease in intestinal motility (Rockstein, 1975). Cross-contamination alone is unlikely to provide a satisfactory explanation for the differences between our rates and those published by Garlick *et al.* (1980). The argument simply does not hold in endeavouring to explain both their consistently higher mucosal rates (119–164%) and lower serosal rates (51–69%), compared with our values (Table 3). In the studies in question, different strains of the rat, raised on different diets, were used, and this may provide a more convincing answer to the apparent discrepancies. Indeed, different rates of growth, and therefore protein turnover, have been clearly shown in different strains of the rat (Waterlow *et al.*, 1978). As far as direct comparisons are possible, we have reported lower rates of synthesis in the liver, heart, skeletal muscles (Goldspink & Kelly, 1984; Lewis *et al.*, 1984) and the small and large intestine (Table 2) of the Charles River CD (Sprague–Dawley) strain of rat, compared with the

London group's work on Wistar rats (McNurlan *et al.*, 1979; Garlick *et al.*, 1980; McNurlan & Garlick, 1980). There are therefore consistent differences, which are probably attributable to the strain of experimental animal used. In addition, dietary factors are known to influence the architecture and turnover rates of the gastrointestinal tract. Although the number of villi in the small intestine does not substantially alter during most of post-natal life (Forrester, 1972; Clarke, 1972), alterations in food supply, toxic dietary substances, vitamin deficiencies, various hormones and anoxia are known to influence the shape, dimensions and absorptive functions of the villi (Creamer, 1973; Peters, 1973) as well as the rates of mitosis (Alpers & Kinzie, 1973), protein synthesis (McNurlan *et al.*, 1979; McNurlan & Garlick, 1981) and cell turnover (Lohrs *et al.*, 1973). Differences in diet could therefore trigger changes in both the mucosal and serosal rates of synthesis, that for the mucosa being the more obvious with respect to possible direct effects exerted by digested products within the lumen.

Meaningful growth rates, and hence indirect rates of protein breakdown, cannot be measured in the mucosa, and therefore in neither the small nor the large intestine as a whole. The changing protein mass would always be grossly underestimated because of the secretory activities of goblet and Paneth cells and Brunner's glands, as well as the prolific extrusion of whole epithelial cells. In contrast, the muscularis externa + serosa consists of more stable cell populations, i.e. mainly two layers of circular (inner) and longitudinal smooth muscle and some connective tissue. The fractional rate of synthesis in this smooth muscle, as in the similarly arranged non-striated muscle layers of the large intestine and lower oesophagus, very much higher (approx. 2–4-fold) than in the various forms of striated muscle (Lewis *et al.*, 1984; Waterlow *et al.*, 1978). The rates in the smooth muscle of the lower gut (Tables 2 and 3) are higher, age for age, than those in the oesophagus (Lewis *et al.*, 1984). Like the differences between fast and slow skeletal muscles, part of the differences in smooth-muscle rates may be linked to the amount of contractile activity within the different areas in the gut. For example, the oesophageal and intestinal muscle layers all participate in propulsive peristaltic-type contractions. However, in addition non-propulsive segmental contractions in the intestine, but not in the oesophagus, are important in mixing the luminal contents and facilitating absorption. Hence it might be predicted that smooth muscles, which are subjected to more frequent and more continuous work loads, might exhibit higher rates of turnover. This would indeed be consistent with the trend of synthetic rates as measured here (Tables 2 and 3). The decreased motility of the gut in old age (Rockstein, 1975) would also be consistent with the falling rates of synthesis (i.e. mg/day and per ribosome, Table 3) and cellular atrophy (i.e. $0.41 \pm 0.03$ to $0.31 \pm 0.06$ for protein/DNA) in the serosa (Table 3) and large intestine (Table 2) between 8 and 105 weeks *post partum*.

*Comparison of the different body tissues*

It is clear from these (see also Goldspink & Kelly, 1984; Lewis *et al.*, 1984) and other (Waterlow *et al.*, 1978) studies that different rates of growth and protein turnover are to be found within the individual tissues of the body during development. Age for age, the fractional rates of synthesis showed a definite trend within the tissues studied, i.e. small intestine > liver > large intestine > kidney > oesophagus (> whole body) > heart and slow-twitch soleus muscle > fast-twitch tibialis anterior muscle. These differences in the synthesis rates can be primarily linked to differences in tissue ribosomal capacities. For example, at 44 weeks *post partum* the fractional rate of synthesis and the ribosomal capacity were respectively 9.4 and 8.2 times higher in the liver (Goldspink & Kelly, 1984) than in the tibialis anterior muscle (Lewis *et al.*, 1984). In contrast, tissue and whole-body rates of synthesis per unit of RNA were generally very similar (except that, on average, these values were higher in the intestinal preparations), and remained so throughout both pre- and post-natal life. The major exceptions to this latter generalization were to be found in many of these tissues immediately before birth (i.e. 20 days of gestation) and in old age (i.e. 105 weeks). These particular developmental features have been discussed in detail elsewhere (Goldspink & Kelly, 1984; Lewis *et al.*, 1984).

Although the fractional rates of protein synthesis decreased in all of these body tissues during development, different patterns of growth and associated changes in protein turnover were clearly apparent. For example, protein turnover in the musculature continued to decline right throughout post-natal life (Waterlow *et al.*, 1978; Lewis *et al.*, 1984). Although present, the same trend was less pronounced in the kidney (Goldspink & Kelly, 1984) and intestine (see above), and in the liver and lymphoid tissue such patterns were barely detectable *post partum* (Waterlow *et al.*, 1978; Kelly & Goldspink, 1983; Goldspink & Kelly, 1984). Clearly such developmental changes within the musculature are largely responsible for the declining fractional rate of synthesis within the whole animal (Waterlow *et al.*, 1978; Goldspink & Kelly, 1984). However, because of the rapid increase in skeletal-muscle growth after birth (i.e. increasing to 70–75% of body protein), there is a clear

developmental shift in the contribution of the musculature to whole-body synthesis, from approx. 20% in the 20-day foetus to 45% in the mature adult at 8 weeks (Kelly *et al.*, 1983). In contrast, hepatic contributions to whole-body synthesis and the protein mass decreased with increasing age. An alternative interpretation of these developmental patterns is that they are not so very different, but rather expressed at different points in time in relation to a particular tissue's functional role within the overall development of the animal. For example, the more dramatic developmental changes in the liver and brain appear to precede birth, whereas in the gut and musculature major changes appear after birth and can still be found late into post-natal life. The principal functions of the latter tissues are, of course, designed for an independent existence beyond the uterus, whereas the former tissues are functionally active at a comparatively early stage *in utero*. The kidney, in fulfilling only a limited physiological role before birth, falls somewhere between these examples.

Our measured rates of growth and protein turnover do not of themselves enable us to distinguish between differentiation (i.e. changing protein profiles resulting from variations in gene expression) and growth itself (i.e. an expansion of existing protein populations); rather they are themselves the consequence of the variable contributions of these processes in relation to time. The simple renewal and expansion of a fixed population of proteins will clearly influence the relative rates of synthesis and breakdown. Changes within the protein profiles will, in addition, influence the absolute rates of turnover, since different proteins are known to possess very different half-lives (Goldberg & St. John, 1976; Waterlow *et al.*, 1978). Differentiation as such will clearly feature more strongly during foetal life. However, as indicated above, it can extend, to varying degrees, into post-natal life, depending on the functional significance of the whole, or part, of the tissue in question. For example, it is known that the cortex of the kidney is much less well differentiated than the medulla at birth; hence the more extensive post-natal development of the former region may possibly be linked with its higher rate of synthesis compared with the medulla (Garlick *et al.*, 1976). The steady state is perhaps the nearest one can get to eliminating the contribution of differentiation, the different half-lives for whole tissues mainly reflecting the renewal of established protein profiles.

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