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Symposium on 'Growth'

Growth in perspective

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The word 'growth' is used in so many ways by investigators that it is difficult, if not impossible, to define it in a way that satisfies everyone. The three main perspectives from which growth is studied might be termed the dimensional, the compositional, and the developmental (or functional). Papers that deal more specifically with each of these views appear elsewhere in this symposium. Although these approaches are useful in focusing our thoughts on specific problems, they are not conceptually exclusive from one another. There are many grey areas where strict categorization is neither valid nor helpful.

The three categories do indicate some of the objectives of growth research. Investigators who are concerned with length, mass, and chemical composition are often interested primarily in the immediate practical implications of their research. Thus, studies in humans often have as their objective the definition of normal growth, so that abnormal patterns can be identified (Fomon *et al.* 1982); while in agricultural practice investigators are interested in the manipulation of body, organ, protein, and fat mass to maximize the economic returns of animal production (see Buttery & Dawson, 1990). Even so, workers in both clinical and agricultural research well recognize that the changes in length, mass, and the chemical composition of the body are both accompaniments of, and preludes to, the attainment of functional maturity. Investigators who approach growth from a developmental perspective, however, are often concerned with understanding the development and maintenance of organ function and tend to view changes in cellular mass as one part of the expression of the cellular phenotype. If we take all these views into consideration, perhaps the fairest, but probably the vaguest, definition of growth is that it is a collection of time-related phenomena that occur between conception and maturity.

The perspectives within each of the three main views of growth are many and varied. To those concerned with nutrient requirements, the definition of normal or adequate growth both in magnitude and composition is important. For example, the amounts of dietary energy and protein used in support of growth over a lifetime vary markedly between species (Table 1, see also Reeds *et al.* 1985), and this is a function of the proportion of life-span occupied by the growth phase. The study of the changes in

Table 1. *Influence of the length of the growth period and the increase in mass between birth and maturity on lifetime protein and energy requirements*

Species	Growth period ÷ life-span	Mature wt ÷ birth wt	'Growth' requirement ÷ total requirement	
			Energy	Protein
Mouse	0·06	30	0·032	0·152
Rat	0·06	40	0·029	0·150
Pig	0·15	100	0·063	0·200
Man (male)	0·25	20	0·002	0·025
Elephant	0·15	40	0·001	0·060

Assuming maintenance energy requirement is 440 kJ/kg body-wt $(W)^{0.75}$ maintenance nitrogen requirement is 140 mg N/kg $W^{0.75}$.

chemical composition that accompany weight and length increments is critical to the formulation of the quantities and patterns of nutrients that must be supplied in the diet to support growth (Spady *et al.* 1976; Fjeld *et al.* 1989). Investigations of the mechanisms that have evolved to regulate organ mass (see Goss, 1990) have substantial implications for the degree to which function can return following traumatic injury or nutritional insult, and on strategies that could be adopted to facilitate recovery (see Burns, 1990).

AGE, LENGTH, AND MASS

The interspecific range of mature body mass in mammals is extremely large (at least 10^8). Given this enormous range, it is remarkable that all embryos start life as a single cell of approximately the same volume (1000 μm^3) and mass and that at implantation the majority of the cells present in the blastocyst will be subsequently involved in supporting nutrient flow from the mother to the fetus rather than in forming the precursors for organogenesis. The body itself then develops from only a limited number of cells.

Because the rate of cell division up to the 64–128-cell stage shows only small interspecific variation, a critical point for the regulation of ultimate body mass might be the period immediately following the appearance of the primitive streak (Snow & Bennett, 1978), and it seems apparent at this time that embryos of different species transiently achieve different rates of proliferation. Even so, the phrase 'a small range' can be very misleading when used in reference to a period of proliferative growth. Very small proportional differences in the cell-doubling time can lead to rapid divergences in mass; a 10% difference in doubling-time would lead to a twofold difference in cell number after only eight cell generations. Thus, minute differences in a genetically determined rate of proliferation (Falconer *et al.* 1981) will have a substantial impact on ultimate cell number and, presumably, body mass. Furthermore, maternal influences, especially as they affect the nutrient supply to the fetus (Table 2) have an important bearing on the rate of growth during the later stages of gestation and the chemical composition of the newborn.

Proliferative growth is not sustained indefinitely at the rates seen shortly after implantation. As development proceeds, both the fractional rate of mass gain and especially the rate of proliferation slow markedly, and hypertrophic mechanisms assume a progressively important role in determining the increases in cell, tissue, and body protein mass.

Table 2. *Body fatness of the newborn of different species in relation to fetal load**

Species	Fetal load†	Fat ÷ body-wt (%)
Mouse	13·4	3·2
Rat	2·8	2·0
Cat	1·0	1·8
Pig	0·4	1·1
Rabbit	0·7	4·0
Guinea-pig	0·2	10·0
Man	0·06	16·0

* Values taken from McCance & Widdowson (1977) and Lodge *et al.* (1978).

† Calculated as $\frac{\text{birth weight} \times \text{litter size}}{(0{\cdot}33 \times \text{gestation period}) \times \text{maternal weight}}$

It is generally held that hypertrophy is of specific importance during post-natal growth. However, studies (Rucklidge, 1981) of newborn mice from strains genetically selected for differences in mature body-weight (Falconer, 1973) have revealed that, despite an expectation that differences in cell number would underlie the differences in body protein mass, the main difference at birth resulted instead from an increase in overall cell size (Table 3). Even so, as the data in Table 3 show, the growth period cannot be divided simply into hyperplastic and hypertrophic phases. Substantial increases in nuclear number occur in all organs in both the prenatal and post-natal periods, and of course some cells (e.g. of the gut mucosa and dermis) continue to proliferate at high rates throughout life.

If we confine ourselves to interspecific comparisons, it seems that although birth has a substantial functional impact on the organism, as far as overall body mass is concerned, birth is merely a point on a line of continuously slowing fractional rates of weight gain and protein deposition. The relationship between mature weight and birth weight is a function of the proportion of the life-span taken to achieve adult stature and mass and the rate at which these are attained. Rodents have high fractional rates of protein deposition immediately after birth (10–20%/d) but they sustain high rates of growth for only approximately 6% of their life-span. Pigs grow at a slower fractional rate (2–4%/d) but do so for a higher proportion of their life-span. The other major milestone as far as growth is concerned is the attainment of reproductive competence, for at this point linear growth either ceases or slows markedly. Rats reach sexual maturity when their weight is approximately forty times that at birth, but pigs reach a similar stage of maturity after a 100-fold increase in body-weight. In this respect, pigs are not representative of mammals as a group. Humans are another major exception; their body-weight has increased by only fifteenfold from their birth weight, even though protein deposition continues for 25% of the period between birth and death.

ORGAN GROWTH DURING DEVELOPMENT

This subject will be considered in greater detail in this symposium by Goss (1990). Broadly speaking, there are three phases of organ growth. The first is the formation of

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Table 3. *Cellularity and cell size in mice selected for different mature body-weights**

Post-natal age (d)	Body DNA (mg)		Body protein (mg)		Protein: DNA (mg/mg)	
	Large	Small	Large	Small	Large	Small
0	5·8	7·4	229	174	39	24
19	11·8	10·9	1767	1231	150	113
42	45·8	30·2	5778	3771	126	124

* Values taken from Rucklidge (1981).

the endodermal, ectodermal, and mesodermal layers (presumably driven by a cell's position within the early embryo and, hence, its microenvironment) and the commitment of these cells to a specific lineage. The second phase is the differentiation of these cells into a primary differentiated state, and the final phase, maturation, is completed only when the organ assumes its full physiological or metabolic function.

The growth and differentiation of skeletal muscle provides an appropriate example of the phases of organ growth. In skeletal muscle, the committed (or determined) stage is the formation of a population of primary myoblasts. Two lines of evidence suggest that, in muscle at least, the formation of the cells is a process of positive determination in that it involves transcriptional activation rather than primarily the suppression of the mRNA synthesis. Recent work has identified two genes, termed myd (myogenic determination gene; Pinney *et al.* 1988) and $MyoD_1$ (myoblast determination gene number one; Davis *et al.* 1987), that are activated sequentially at the initiation of determination. When transfected into other cells, they commit the recipients to express genes that are characteristic of myotubes (Davis *et al.* 1987). Work with heterokaryons (summarized by Blau *et al.* 1985), supports this evidence by showing that the presence of muscle-specific gene products (presumably those of primary 'differentiation genes') activates the transcription of muscle specific genes in the non-muscle partners, even when the non-muscle cell has already been committed to its own pathway of differentiation. Even so, in the experiments of Blau *et al.* (1985), the highest frequency of muscle-gene expressing heterokaryons were those formed from myoblasts and differentiated cells of a mesodermal origin, which suggests that even at very early stages, cells are predisposed to a particular line of differentiation.

In skeletal muscle, unlike many other types of cells, differentiation coincides with a withdrawal of the myoblast nuclei from the cell cycle. It is at this stage that the cells begin to fuse into multinucleated myotubes. Current hypotheses posit that these events are initiated primarily by interactions of the protein products of the two or three differentiation genes (myd and $MyoD_1$ and perhaps myogenin) with the promoter-enhancer regions of some (e.g. muscle-type creatine kinase and α-actin), but not necessarily all, genes whose products define skeletal muscle. Even so, in the fetus, interactions between fibroblasts, myotubes, and the growing skeleton lead to the formation of the tendon attachments, and ingrowth of fetal axons initiates further differentiation processes. Although the differentiation processes can be influenced by culture-specific extracellular matrices (e.g. laminin; Foster *et al.* 1987), specific cells (especially fetal neurons; Brodie & Sampson, 1987) and peptide trophic factors (Florini, 1987) it is important to recognize that these extracellular factors promote the early events of differentiation but are not prerequisites.

At birth, the skeletal muscle of altricial mammals is in many respects close to, the end of the primary stages of differentiation. In other mammals, however, further development has occurred *in utero*, as witnessed by the fact that many mammals are mobile at or very soon after birth. Thus, the presence of primary and secondary myotubes is the starting point for the later processes that must occur before skeletal muscle becomes fully functional, i.e. before it exhibits externally regulated (as opposed to spontaneous) contractions. These processes are followed by the time-ordered expression of three myosin isoforms and the eventual appearance of histochemically and phenotypically distinct fibre types. Some, but not all, of these events are strongly dependent on the pattern of innervation by the maturing motor nervous system.

At the mechanistic level, these end-stages are continuations of differentiation, but in terms of function, a better term might be maturation, a term that can be applied to other complex tissues. For example, in newborn rodents, the major structural elements of intestinal differentiation are present, but once the animal is weaned, the expression of brush-border enzymes and certain nutrient absorption mechanisms (especially those that are dependent on pancreatic secretion) are quite different (Henning, 1986).

GROWTH AND FUNCTION

Throughout life, the mass of many organs alters in response to changes in the physical or 'metabolic' work that each is called upon to perform. Such alteration in response to workload has been observed in the skeleton (see Goss, 1986), skeletal (Laurent *et al.* 1978) and cardiac muscles (Fanberg & Posner, 1968; Morgan *et al.* 1980), the liver and kidney (Ferrell & Koong, 1986), and the small intestine (Ferrell *et al.* 1986; Table 4). In skeletal muscle, phenotypic expression reflected in fibre and myosin types is also sensitive to functional load (Gregory *et al.* 1986).

However, although the mechanisms (hyperplasia or hypertrophy, or both) that underlie such increases in mass generally reflect those in effect during developmental growth, the extent to which functional demand affects the regulation of developmental growth itself is a separate question.

It is not surprising that organs such as the liver and heart, which play a vital role in the fetus contribute more to body protein mass at birth than at later stages of development. Although other systems (e.g. intestine and skeletal muscle) grow *in utero*, their major growth occurs after birth, when their function has become vital to the maintenance of life. Thus, in the rat the proportional contribution of the small intestine to body mass increases by at least twofold between birth and weaning, and over the same period the fractional rate of leg muscle protein increase exceeds that of the body as a whole by a factor of approximately four (Davis *et al.* 1989). A general relationship, therefore, does seem to exist between the allometry of organ growth and functional demand.

On the other hand, the long bones appear to have a genetically determined predisposition to achieve a given length irrespective of the growth status of other organs (see Goss, 1986). Even so, while length-growth essentially proceeds normally in transplanted fetal long bones, bone diameter and particularly the degree of calcification (the other functionally important aspects of bone growth) are sensitive to weight loading.

The division of growth regulation between genetic–temporal and functional aspects becomes important when we consider the degree to which growth and development are influenced by nutrition. During fetal life protein deposition seems to be significantly less

Table 4. *Influence of the level of immediately preceding feed intake on gastrointestinal and liver mass in growing lambs**

Preceding intake (g/kg body-wt)	Liver wt (g/kg body-wt)	Intestinal wt (g/kg body-wt)
14·6	13·4	41·9
23·8	13·9	40·7
34·5	16·2	53·0

* Values taken from Ferrell *et al.* (1986).

Table 5. *Relationship between milk intake, protein and fat deposition in rats of two ages suckled in different litter sizes (ten and sixteen pups per dam)**

		Milk intake (/rat per d)		Deposition (mg/rat per d)	
Age (d)	Litter size	kJ	mg protein	Protein	Fat
5	10	39	302	200	220
	16	31	246	200	20
Difference (%)		(−20)	(−18)	(0)	(−91)
15	10	78	904	400	350
	16	48	653	270	140
Difference (%)		(−38)	(−27)	(−32)	(−60)

* Values of M. L. Fiorotto, D. G. Burrin, M. Perez, D. N. J. Reeds and P. J. Reeds (unpublished results).

sensitive to nutritional deprivation of the mother than at later stages of life. In rodents, the insensitivity of fetal protein deposition to maternal nutritional deprivation seems to be carried over into early post-natal life (Table 5). However, body protein is merely the sum of the protein in the different organs and in the rat early post-natal protein deposition is dominated by that in muscle. The second week of life in the rat is marked by a particularly intensive phase of muscle growth and maturation. At weaning, when the change to an adult pattern of gene expression is virtually complete, muscle growth becomes progressively more sensitive to undernutrition. It may, therefore, be the nature of the maturation processes that occur at any given time, rather than the presence or absence of proliferation, that influences the sensitivity of protein deposition to nutrient deprivation. A similar conclusion can be drawn from studies in early weaned piglets (Seve *et al.* 1986), in which, at low intakes, dietary protein utilization seems to be diverted specifically to the gastrointestinal tract rather than to skeletal muscle. It is perhaps no coincidence that pigs are born with well-developed musculature, but when they are weaned, intestinal development becomes crucial.

BODY AND TISSUE CHEMICAL COMPOSITION

There are many ways to visualize body composition (see Fuller *et al.* 1990). Classically, body composition has been investigated from three perspectives:

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(1) as water spaces (total, intracellular, extracellular, intravascular, and extravascular compartments),
(2) as energy-expending and energy-storing compartments (lean body mass, fat-free body mass, fat mass, or simply as body protein and fat), and
(3) in terms of elemental composition (organic, inorganic, and nitrogen masses).
As organ and, hence, body mass increases during development, a temporal sequence of changes is also initiated in the relationships between these various experimentally defined compartments, including the elemental composition of the body (Widdowson & Dickerson, 1964). For example, the hydration of the body as a whole decreases, and the distribution of this water between intracellular and extracellular compartments changes in favour of intracellular compartments.

It seems true that, for whatever mechanistic reason (Millward, 1988), immature mammals will seek to use their nutrients to enable a rate of protein deposition as close as possible to their genetic maximum. After birth, the rate of protein deposition is primarily dependent on the degree to which the dietary protein supply (both in terms of quantity and amino acid pattern) allows the genetic programme to be expressed. From a compositional viewpoint, the rate will also be determined to some extent by the volume of cells available to 'store' this protein (the 'bag hypothesis' of cellular growth; (see Millward, 1988), while at the same time the overall pattern of protein deposition will be subject to the previously mentioned 'organ hierarchy'.

The changes in body composition that are shown in weight–water relationships, therefore, reflect both dimensional growth and chemical maturation. Thus, increases in overall cell dimensions, driven in part by previous accretion of cellular protein, effectively exclude extracellular water, while at the same time the increase in cellular protein occurs at the expense of cell water. The relationship between cell protein and cell water eventually reaches a stable value, which at maturity shows little interspecific variation. In immature mammals, therefore, the nitrogen:weight ratio of the fat-free body mass should really be viewed as an index of their relative 'chemical' maturity (Table 6).

At later stages of development, however, the analysis of changes in body water–body-weight relationships is complicated by the fact that, as the organism approaches functional maturity, tissue growth and, hence, protein deposition slow, and fat deposition makes a progressively greater contribution to weight gain. We do not know whether this change reflects an active up-regulation of fat storage or merely an increased availability of organic nutrients above that needed to support protein deposition, and it is an important question in its own right. From a nutritional point of view, however, as maturity is approached, the energy stored per unit weight gain increases, because fat deposition involves little accompanying deposition of water.

It is important to recognize, however, that the changes in body composition typical of an animal approaching maturity can also be produced in an immature animal by suitable manipulations of nutrient intake. Thus, no clear relationship exists between the magnitude of the fat stores of the newborn and its maturity (measured as the proportion of birth weight to mature weight), but a relationship does exist between what we have termed the fetal load and fat mass at birth. A fetal guinea-pig or human, therefore, (each born with substantial fat stores), present the mother with a relatively low fetal load, but a fetal rat or mouse in which the conceptus may approach 40% of maternal weight, is born with a very low fat mass at birth. Fat deposition then is largely a reflection of the degree

Table 6. *Body composition of the newborn of different species in relation to 'maturity'**

Species	Birth wt ÷ mature wt (%)	Protein ÷ fat-free wt (%)
Rat	1·6	10·0
Rabbit	1·9	11·2
Pig	<1·0	11·7
Cat	3·4	13·1
Man	5·0	14·5
Mouse	6·6	14·4
Guinea-pig	15·8	18·7

* Values taken from McCance & Widdowson (1986) and Rucklidge (1982).

to which energy intake exceeds the energy expenditure associated with the maintenance of physiological viability and protein deposition.

It is possible, then, to discern a series of genetically and developmentally regulated priorities for growth. We have already pointed to a hierarchy of tissues whose susceptibility to nutritional insult seems to reflect the relative importance of inherent and functionally regulated growth at a given age. The hierarchy in turn reflects the contribution of each organ to overall viability and, hence, to the pace at which the organ is maturing. The pattern is similar in the broader aspects of body composition. Nutritional deprivation primarily depletes the body of fat, then protein, and these changes are reflected in changes in the extracellular and intracellular water spaces. Realimentation reverses the depletion with a rapid accretion of water, followed by protein and then, as energy intake allows, the deposition of fat (Patrick *et al.* 1978).

It is almost easier to answer the question 'what is growth?' by stating what it is not. It is not simply an increase in body dimensions, because this definition conceals a complex pattern of changes in organ mass and phenotypic expression that lead to the attainment of functional maturity. Nor is growth merely an increase in mass, because this definition conceals changes in chemical composition that reflect the maturity of the organism and its nutritional status. Perhaps no single all-encompassing term exists to describe a multifactorial process whose only common denominator is the passage of time.

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