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Review Article

Skeletal Muscle Cell Mass and Growth: The Concept of the Deoxyribonucleic Acid Unit

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Speculation

Two important predictions, contradicting current opinion, arise from this review:

Removal of the pituitary causes loss of muscle deoxyribonucleic acid (DNA) in the weanling rat. Exercise causes increase in muscle tissue with a commensurate increase in DNA and protein. Therefore, some of the muscle DNA must be in a dynamic state.

Calorie restriction (without protein restriction) imposed during the postweanling period of growth in rats does slow cell multiplication but does not cause permanent growth retardation. Therefore, protein restriction, *per se*, is probably responsible for permanent growth retardation in the experimental animal.

Introduction

Muscle contains a high proportion of the cellular mass of the body and is of considerable importance to protein synthesis, especially in larger mammals. It is the purpose of this paper to review past and new information concerning the growth of skeletal muscle and to support and substantiate the concept of the DNA unit in muscle. This essentially functional concept assumes that each nucleus within the fiber has jurisdiction over a finite mass of cytoplasm.

Because the DNA per diploid nucleus is constant, the nuclear population of the muscle mass can be appraised if the sample analyzed is representative. Data will be presented supporting this statement, and methods of measuring muscle mass will be discussed. Moreover, the ratio of protein to DNA should reflect the size or mass of such a functional cell unit. Cell size can then be compared with organ size or, in this instance, total muscle mass.

Mathematical expressions are presented derived from ongoing work concerning increments in the size and number of such functional muscle units when they are related, respectively, to chronological age and body size during development.

The functional DNA unit is influenced by nutrition, hormones, and exercise. We believe postnatal cytoplasmic growth of muscle tissue is influenced by protein intake and insulin activity while growth hormone and calorie intake influence the rate of increase in the number of nuclei. Alterations in the production and tissue response to these hormones can be detected by muscle analysis. Since satellite cells would appear to be progenitors of myoblasts, factors that influence growth must influence satellite cell behavior.

Developmental Information

The musculature is made up of special cells—muscle fibers. Muscle fibers arise from myoblasts which originate in the middle germinal layer of the embryo (mesenchyme) [10, 100, 103, 128].

Myotubes form by the fusion of multinucleated cells, and the nuclei within the myotubes are thought not to divide mitotically or amitotically [4, 123]. During the last trimester of pregnancy muscle nuclei of the human fetus move to the periphery of the cell (or are displaced by the myofibrils). Further postnatal growth was assumed to occur by increase in fiber size, or length, or both [82].

Surrounding an entire muscle or smaller muscle bundles or the myofiber are discrete connective tissue

sheaths, and chemical analysis reveals only 1% collagen/g fresh weight [19]. Cells such as histiocytes, fibroblasts, neuronal cells, and adipocytes are present in muscle tissue and contribute by virtue of their nuclei to the DNA content of each gram of muscle. Work in progress by Dr. Charles Friedman in our laboratory indicates that during growth 25% of the nuclei in human muscle are outside the myofiber. The method used for this determination is that of Dunnill [37].

Satellite cells according to Mauro [88] are mononucleated cells that are wedged between the basal or plasma membrane of the muscle fiber and the myotubule. During embryonic life these cells are abundant; however, it is not clear whether they do or do not participate in normal myogenesis [70, 105].

Reznik [111, 112] found that satellite cells are more numerous in areas undergoing regeneration and that these cells incorporate tritium-labeled thymidine. He considered them "stem cells" capable of providing myoblasts for the mature mammal and for regeneration of skeletal muscle tissue.

Bischoff and Holtzer [5] recently found that myotubes formed by the union of myoblasts involved cell recognition and membrane-to-membrane interactions when the cells were in the G1 phase of division. They also recognized that certain surrounding stem cells remained and suggested that these cells were "prime candidates for the recruitment of new myoblasts during regeneration." They observed that satellite cells could constitute presumptive myoblasts.

A review by Kelly and Zachs [74] embraces many studies showing that secondary and tertiary muscle cells originate as buds from the walls of primary cells during fetal life; others found that a tertiary generation of muscle cells develops from mononuclear cells in close association with primary generations of myotubes [2, 32, 34, 93, 98]. Kelly and Zacks [74] emphasized the continued presence of undifferentiated cells between myotubes that have been described by Mauro [88] as being "fibroblast like." It is these undifferentiated cells, which lie beneath the basal lamina surrounding the myotubes, that can be classed as belonging to the potential replicating population. Several investigators recorded that the frequency of satellite cells in muscle declines as the fetus reaches term. Such cells, according to Wirsen and Larsson [136], are responsible for the checkerboard pattern of distribution of histochemically distinct myofibers when muscle is observed under the microscope.

Shafiq *et al.* [116] concluded that undifferentiated myoblasts and satellite cells are so close to the myofibers that their nuclei appear, by light microscopy, to be within the myotube. These workers noted that mitosis occurred only in cells outside the myotube and not in any cells that had evidence of myosin synthesis. According to Lee [81] and to Klinkerfuss [75] the plasma membrane surrounding satellite cells can penetrate into the sarcoplasm and additional nuclei are added to the myofiber.

Satellite cells would appear to be of great importance to the understanding of muscle growth and to be responsible for the increments in DNA of muscle during growth. Presumably they are the progenitors of the myoblast.

The ultrastructure of striated muscle has been reviewed [118] and will not be discussed here except to point out that arguments relating to the limits of the extracellular volume in muscle [127, 129, 130] should take into account the sarcotubular system [69].

Histological Approaches to Quantitation of Fiber Size and Number

In 1898 MacCallum [82] studied the middle third of sartorius muscle in the adult, newborn, and fetus; he found in the adult a cross-sectional fiber area nine times that of the newborn. An increase in muscle fiber volume occurred after 6 months of gestation, but no increment in the number of muscle fibers or in the number of nuclei. In 1902 Godlewski [50] reached a similar conclusion, whereas Schiefferdecker [114] insisted that the number of nuclei in muscle fibers increased postnatally.

Tello [125] considered that new fibers probably do arise in the human postnatally or prenatally. Montgomery [97] found that in the fetal period in humans the number of fibers (and nuclei) increased, and that between week 32 of gestation and 4 months of age, the number of muscle fibers doubled; between birth and adulthood, the number of nuclei in the cross-sectional areas studied increased by a factor of 10 or more. Concurrently, muscle fiber diameter increases postnatally two- to threefold [9, 36, 59, 137].

In other mammals, up to a 10-fold increase in fiber diameter has been shown [29, 51, 60, 63, 73, 121]; increments in sarcomere units [89, 99, 117] are in the same order.

The mere measurement of fiber diameter, however, throws little light on the actual increase in fiber mass, which is three-dimensional. Inspection of body composition changes in the mouse during growth [22] and consideration of the changes in muscle fiber length and sarcomere number [52] show clearly that sarcomere

number increases in male mice with the adolescent spurt, while muscle fiber length correlates well with the overall growth of the lean body mass. Thus, measurement of fiber diameter or cross-sectional area yields very limited information.

The weight of evidence indicates that new fibers can arise after birth, and the old thesis that no new nuclei appear in muscle postnatally is certainly erroneous.

Muscle Mass

Muscle mass and body weight; individual muscle weight. In cattle, sheep, and pigs [60, 90, 106], the weight of a dissected individual muscle, or anatomically defined group of muscles, is closely correlated with the total muscle mass. Butterfield [12] showed, in addition, that age, weight, or breed of animal had no bearing on the relations obtained. These results are interesting considering the striking differences in the external shape of the different types of cattle studied. Thus, it is feasible to predict with accuracy the muscle mass of an animal by estimating the weight of an individual muscle which is anatomically defined.

Latimer [79] showed that muscle mass in the fetal cat was linearly related to fetal weight during gestation. Jackson and Lowrey [72] found that muscle mass in albino rats was 20–25% of body weight in the newborn; at weaning, 26–30%; at sexual maturity, 37–43%; at 5 months, 41–44%. No sex differences were demonstrated. Caster *et al.* [13], using gravimetric methods, dissection, and actomyosin determination, found a muscle mass of 45% of the body weight in rats of 320–350 g.

Cheek *et al.* [24, 58] determined the muscle mass in male eviscerated Sprague-Dawley rats without skin or feet (defined as carcass), using three methods which gave comparable results. They showed that the relation between muscle mass and body weight in rats can be clearly defined by linear equations (Table I). In this study muscle mass constituted 80% of fat-free carcass, and a similar equation has evolved (Table I) for prediction of muscle mass from fat-free carcass.

Welcker and Brandt [132] reviewed the literature up to 1912 on the growth of human muscle mass. The newborn baby was found to have a muscle mass of 23–25%, while the adult had a value of 43.2% of body weight. These data agree with recent observations reported about dissections of the human body [47].

Muscle mass and protein stores. Perry and Zydowo [108] found that ribonucleoprotein particles associated with endoplastic reticulum were not numerous and suggested that muscle was not a large contributor to protein synthesis. As one proceeds from the smallest mammal to the largest, however, mass of visceral tissue (for example, liver) becomes less relative to body weight, while muscle mass remains, for the most mature mammals, from 40 to 45% of body weight. Differentially, in the larger mammals, muscle mass is of increasing importance with respect to protein synthesis [101]. Martin and Fuhrman [87] demonstrated that the visceral mass of large animals accounts for a lesser proportion of the total oxygen consumption than that in smaller mammals. Consideration by Munro [101] of the ratio of liver mass to muscle mass plotted against body weight shows that liver weight does not increase in proportion to weight of the mammal and that muscle mass remains at 45%. Thus, the proportion of liver mass to muscle mass is much less for the elephant than for the mouse.

Muscle protein and metabolically active protoplasm. Chinn [30] studied male and female rats from 80 to 190 days of age. He emphasized that muscle protein was a constant percentage of total body protein in the mature animal and that this determinant correlated highly with excretion of creatinine in urine. Predictions could be made of muscle protein and total body protein from the determination of total body potassium. It is clear from his data that muscle protein constitutes more than half of the total body protein.

Table I. Data on rat muscle

Cell size
Individual cell mass (ICM), in pg *vs* muscle mass (MM) in g:
♂ ICM = 22.52 + 156.19 (MM) − 1.819 (MM)^2 + 0.00853 (MM)^3
SD is 698 pg and N = 54
(Age range 14–150 days)
♀ ICM = −184.22 + 184.34 (MM) − 0.88 (MM)^2
SD is 764 pg and N = 16
(Age range 21–98 days)
Protein/DNA ratio (PD), in mg/mg *vs* muscle mass (MM), in g:
♂ PD = 17.9 + 5.14 (MM) − 0.056 (MM)^2 + 0.00027 (MM)^3
N = 65 SD = 26
(Age range 2–154 days)
♀ PD = −17.3 + 8.28 (MM) − 0.0425 (MM)^2
N = 19 SD = 30.6
(Age range 21–98 days)
Muscle mass (MM), in g *vs* weight (WT), in g or fat-free carcass (FFC), in g:
♂ + ♀ MM = −6.645 + 0.387 WT
N = 112 SD = 5.49
r = 0.992
(Age range 2–154 days)
♂ + ♀ MM = −4.16 + 0.81 (FFC)
N = 112 SD = 3.19
r = 0.997
(Age range 2–154 days)

Earlier work by Cheek and West [26, 27] showed that with male albino rats at 8 weeks of age (220 g body weight), there were 19.5 g of muscle protein and 22 g of protein outside muscle. It was also shown [28] that total body collagen accounts for 12 g protein in this rat. The vast majority of this amount of protein resides in skin and skeleton; therefore, as much as two-thirds of the metabolically active protoplasm resides in the muscle of the intact rat.

When considered as a fraction of total body potassium in the human, the potassium content of muscle varies with growth. After 10 years of age, muscle tissue in the male accounts for increasing amounts of body potassium, and at 17 years of age, two-thirds of body potassium is present in muscle mass [39]. It would seem reasonable to believe that metabolically active protein would have a similar distribution.

Muscle mass and excretion of creatinine in urine. The skeletal musculature accounts for 25–40% of body weight from infancy to maturity. Assessment of muscle mass in the living mammal has most often been made by estimation of 24-hr excretion of creatinine in urine. Creatinine is the cyclic anhydride of creatine. Most of the creatine formed is found (as creatine phosphate) in muscle tissue, where a portion of it is continually converted to creatinine, this conversion being essentially irreversible [8]. Creatinine in the urine of animals maintained on a creatine/creatinine-free diet is directly derived from tissue creatine [6]. Shaffer, in 1908 [115], first drew attention to the relation between excretion of creatinine and muscular development, and Folin [46] and Deuel *et al.* [35] showed that creatinine excretion is independent of nitrogen excretion. Older [66, 94] and more recent reviews [1, 57, 85, 107, 131] discussed the issue of creatinine excretion. Misinterpretation, particularly in the human, of the usefulness of studying creatinine metabolism in relation to muscle mass has occurred because of poor control of diet, imprecise urine collection, and methods of determination [78]. Garn [48, 49] emphasized that for herbivorous animals excellent correlations between creatinine excretion and other determinants of body composition should allow the use of creatinine as a direct measure of muscle mass. Figure 1 illustrates the relation between creatinine excretion in children and male infants with height (as a measure of maturational age) when a low creatine/creatinine diet is instituted.

Meador and co-workers [91] have recently demonstrated, using 1-^{14}C-creatine as a tracer, that 93.6% of body creatine of rats is in skeletal muscle. Borsook and Dubnoff [8] considered that 98% of body creatine is in skeletal muscle. Since at least 90% of creatine exists in muscle mass, the injection and distribution of labeled creatine provides another method for this determination [91].

Because creatinine excretion reflects muscle mass, it is of importance to know how much creatinine is equivalent to 1 kg of muscle mass in different mammalian species. Chinn [30] studied muscle protein (or muscle mass) creatinine and creatine in the same ani-

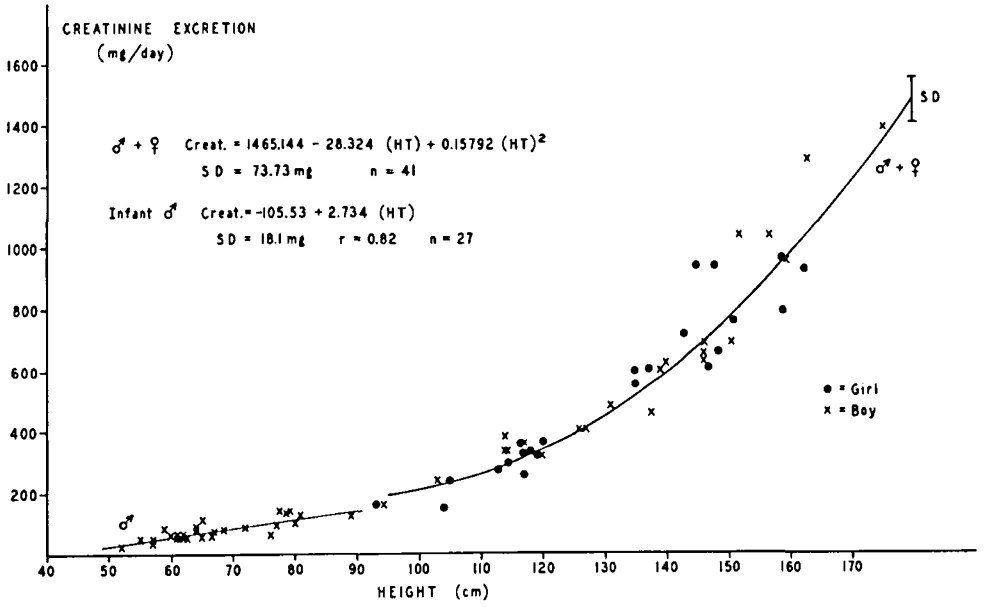

Fig. 1. Creatinine excretion is plotted against body length for infants and children up to 17 years. The relation is linear for infant boys and quadratic for boys and girls from 4 to 17 years with no sex differences demonstrable for the latter groups.

mal. Assuming that 200 mg protein are present in 1 g muscle, the mean muscle mass for the mature rats studied was 131.8 g. Thus, 1 g creatinine excreted/24 hr was equivalent to 20.5 kg muscle mass; *i.e.*, factor $F = 20.5$.

Graystone [57], assuming that 56.9% of the total intracellular water contained in the muscle mass of rats was similar in value for the human, calculated in 36 normal children that for 17 boys and 19 girls $F = 21.2 \pm 3.5$ and 20.7 ± 4.05, respectively.

On the basis of these considerations, Cheek and coworkers have used a creatinine equivalent of 20 for simplicity in the calculation of muscle mass [16, 19, 57].

In Figure 2, the intracellular water (total body water − extracellular volume) of the body has been plotted against creatinine excretion for normal children 4–17 years and for male infants 0.2–2 years. The two linear equations are shown for the children. Since no sex difference was found by analysis of covariance, no separation has been carried out. The slopes of the lines for the two equations are different. If the creatinine excretion is converted to muscle mass using the factor of 20, it becomes clear that an infant excreting 100 mg creatinine/24 hr has a muscle mass of 2 kg and (from the graph) an intracellular water of 3.5 liters. Previous work showed that 50.6% of the fresh fat-free muscle in these infants was intracellular water. Hence, the total intracellular water of muscle would be 1.012 liters or 28.9% of total intracellular water. Since the ratio of cell water to cell protein is a constant, these percentages represent cell mass relations. Examination of the older children reveals, by a similar approach, that the intracellular mass of muscle, as a proportion of total intracellular mass, would rise from 35 to 69% from 4 to 17 years.

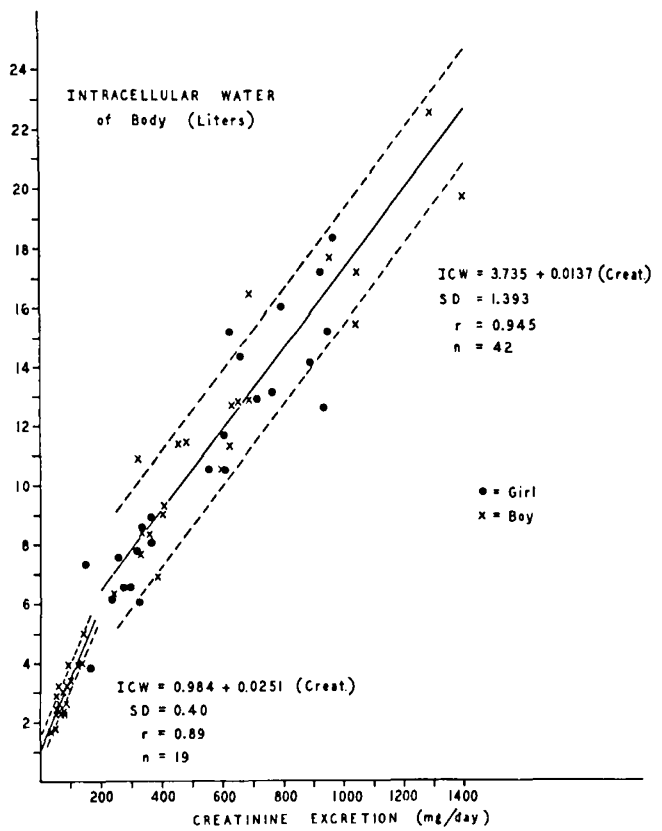

Fig. 2. The intracellular water (D_2O space minus corrected bromide space) is plotted against creatinine excretion for infant boys 0.2–2 years and for boys plus girls 4–17 years. From this figure and available information one can calculate the ratio of intracellular water in muscle to total intracellular water. At 17 years muscle cell mass constitutes 70% of total intracellular mass.

The relations between intracellular water and body weight have a constant slope for infants and older children up to 17 years [15a], but muscle mass shows a changing relation with body weight or intracellular water. For body weight our present data indicate muscle mass to be 22% in the infant, 27% at 4 or 5 years, and 43% in late adolescence. This is in agreement with the estimation of muscle mass in newborns and adults derived from dissection studies as recorded by Malina [85]. Such values are in accord with data of other investigators [33, 104, 122] concerning creatinine excretion.

It is clear from the above discussion that in mature rats and humans, intracellular mass in muscle constitutes two-thirds of the metabolically active protoplasm. In animals, individual muscle groups correlate strongly with the amount of muscle mass, and chemical analyses allow the determination of the amount of this tissue with precision [20, 56, 131].

The DNA Unit—A Functional Unit

Epstein [42] has shown that the degree of polyploidy in liver is commensurate with the amount of cytoplasm. Thus, a tetraploid liver cell behaves as two diploid cells in terms of mass composition. A hepatocyte containing two diploid nuclei has double the amount of cytoplasm of a single diploid cell. By analogy, it would seem reasonable to believe that one set of chromosomes would dominate a finite volume of cytoplasm. Since muscle is a syncytium, this is essentially a functional concept. During growth, protein per unit muscle reaches a stable level early in postnatal life, so that changes in protein/DNA depend mainly on DNA

differences and the number of nuclei distributed along a given muscle fiber, which in turn must be influenced by hormones and by nutrition.

The DNA unit in rat muscle. The discovery of the constancy of DNA per nucleus [7, 95, 126] (about 6.2 pg/diploid nucleus) allowed the estimation of the number of nuclei in muscle. Mitoses have been observed at all stages in striated muscle in rats [83] and while 1.2–1.7% of nuclei enter mitosis each day in the tibialis anterior of 30- to 100-g rats, polyploidy does not occur [41].

Enesco and Puddy [41] studied histologically the biceps brachii, extensor carpi radialis longus, gastrocnemius, and tibialis anterior muscles in Sherman rats. These rats were 15–94 days of age and weighed 27–320 g. The DNA content increased 2-fold in the biceps and 3- to 4-fold in the other muscles, while muscle weight increased from 9-fold for the biceps to 21-fold for the gastrocnemius. No efforts were made to remove connective tissue layers. The endomysial and perimysial nuclei were thought to constitute about 35% of the total nuclei. Histological counts showed that the number of nuclei within the fibers doubled in the biceps brachii, tripled in the tibialis anterior, and quadrupled in the gastrocnemius and extensor carpi radialis longus over the period of study. An interesting point not mentioned was the fact that, while the number of nuclei and muscle weight increased in different degrees for the different muscles, the ratio of muscle weight per nucleus was reasonably uniform for three of the four muscles. Except for the small muscle, extensor carpi radialis, the other three showed a 5- to 6-fold increase in this ratio from 16 to 86 days of age.

Cheek *et al.* [23] took quadriceps muscle from rats (1–8 weeks of age), taking care to keep connective tissue to a minimum (1%), and found that there was a 500% increment in the DNA of the total muscle mass, assuming the muscle sample to be representative of the whole musculature.

Calculation of the volume of intracellular water in the muscle mass and adding protein (not including collagen) within the muscle mass show that the intracellular mass of muscle increases from 2.4 to 85 g from 1 to 8 weeks. The amount of DNA present can be considered to be mainly in the muscle fibers, and since the nuclei contain a constant amount of DNA, the mass of protoplasm associated with each nucleus is found to change from 777 to 5700 pg from 1 to 8 weeks. This amount of protoplasm (protein plus water) within the cellular phase can be considered to be associated functionally with each nucleus (or unit of DNA). Hence, although histologically each muscle fiber may contain 100 or more of such units, the term "individual cell mass" can be justifiably coined.

A more detailed study of the developing rat [15a] provided evidence that the individual cell mass from 1 to 14 weeks increases 10-fold for males with a period of deceleration from 6 to 8 weeks when the number of nuclei increase owing to adolescent growth. In female rats the growth of the individual cell mass is more accelerated than for the male from the time of weaning. The greatest difference is at 9 weeks; however, by 12 weeks the value for the male is equal to that of the female. Subsequently, cytoplasmic mass per unit DNA is greater for males. At 22 weeks a value of 11×10^3 pg is reached for the male.

The female rat in early postnatal life has larger muscle cells for the same muscle mass. It appears that the male, however, eventually has larger muscle cells.

Inspection of the growth of muscle cell size against time is profitable, but of equal or greater moment is the inspection of individual cell mass against organ size or muscle mass. Table 1 shows a cubic equation for male rats during growth, while for the female a quadratic equation holds.

Since the ratio of cell protein/water is a constant, it can be deduced that the protein/DNA ratio is also an index of cell size (Table I). Figure 3 shows the relation between this ratio and muscle mass. Again, a quadratic equation is described for the female from 3 to 14 weeks and a cubic equation for the male from 2 days to 22 weeks. An accelerated increase in muscle cell size rela-

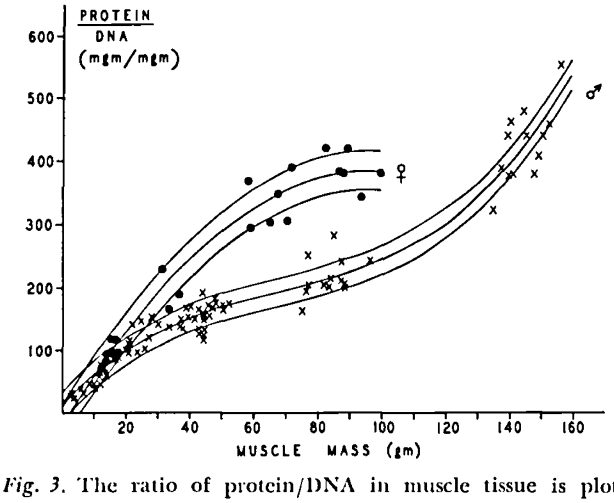

Fig. 3. The ratio of protein/DNA in muscle tissue is plotted against the muscle mass for male and female rats during growth. Note that the relationship is quadratic for females and cubic for males (Table I). The standard deviations are shown. A sex difference in the growth of muscle cell size is thus revealed.

Table II. Concentration of deoxyribonucleic acid and protein in individual muscles of rats[1]

Age, days	DNA and protein, mg/g	Stochastic variables	Muscle (a) Hamstring	(b) Gastrocnemius	(c) Psoas	(d) Erector spinatus
35	DNA	Mean	1.31	1.33	1.33	1.46
		SD	0.16	0.14	0.19	0.16
		N	10	10	10	10
	Protein	Mean	177.4	176.9	174.6	164.9
		SD	7.6	7.5	4.0	9.5
		N	10	10	8	10
49	DNA	Mean	1.01	1.01	0.94	1.08
		SD	0.13	0.14	0.16	0.15
		N	10	10	10	9
	Protein	Mean	193.6	194.9	192.0	183.8
		SD	13.3	6.9	8.7	5.6
		N	9	10	9	10

[1] Statistical treatment by paired analyses showed no differences for the chemical data relating to three muscle groups. The erector spinae muscle showed differences: At 35 days: DNA (*d*) *vs* (*a*) $P < 0.005$ (*c*) $P < 0.05$; protein (*d*) *vs* (*a*) $P < 0.005$, (*b*) and (*c*) $P < 0.01$. At 49 days: DNA (*d*) *vs* (*c*) $P < 0.005$; protein (*d*) *vs*. (*b*) $P < 0.005$, (*c*) $P < 0.05$.

tive to body size or muscle mass occurs in the female. A sex difference in muscle growth is clearly defined. For the same muscle mass the female and male have a different protein/DNA ratio. As body size and muscle mass increase for the male, so does the protein/DNA ratio. Eventually the male possesses a greater muscle mass, and the protein/DNA ratio is greater.

The prediction of the number of nuclei in the entire musculature from a single sample depends on whether the sample is representative. Table II contains data on DNA and protein content of various muscle groups of 38- and 49-day-old rats. Clearly, agreement does exist except for the erector spinatus muscle where larger amounts of collagen are present. In general, the ratios of protein/DNA are consistent between muscles for an individual rat. Hence, these findings are different from those of Enesco and Puddy [41].

The number of nuclei present in the entire musculature of the rat can be found by the expression: DNA per gram of muscle × grams of muscle mass. Division by 6.2 pg yields the number of nuclei. Figure 4 depicts the relation for male and female rats against time or age. This is the appropriate base line since cell division is time-dependent. At 1 week, rats have 2.5×10^9 nuclei in the muscle mass. Males at 7 weeks have 12.5×10^9 nuclei (about a fivefold increase), while females at the same age have 6×10^9 nuclei (probably a twofold increase if the pattern of growth prior to weaning is the same for the male and female). At 14 weeks, the male rat appears to have a constant number of nuclei of about 13×10^9, but the female only reaches 8×10^9. Again, a sex difference is revealed. For males the increment in the number of nuclei is progressive up until 50 days when sexual maturation occurs. During this latter period an adolescent spurt is defined until a plateau is reached or the "steady state of cell population" [80].

Interestingly, castration of the female rat at 3 weeks causes a pattern of cell growth characteristic of the male, so that at 14 weeks no statistical difference is found between the castrated female and normal male [15b]. Cell size, however, does not reach that of the male.

Gordon *et al.* [55] studied the growth of quadriceps muscle of male Sprague-Dawley rats from day 43 to 155. The rats were already approaching sexual maturation, and there was a 2.5-fold increase in DNA content of muscle with a stable value at 90 days of age. Sarcoplasmic and myofibrillar protein continued to increase until 150 days. The general observations are in agreement with our own. Indeed, appraising their data on muscle mass/body weight, it is possible to show a similar number of nuclei for age.

Munro and Gray [102] made a comparative study of DNA, RNA, and protein content of muscles for var-

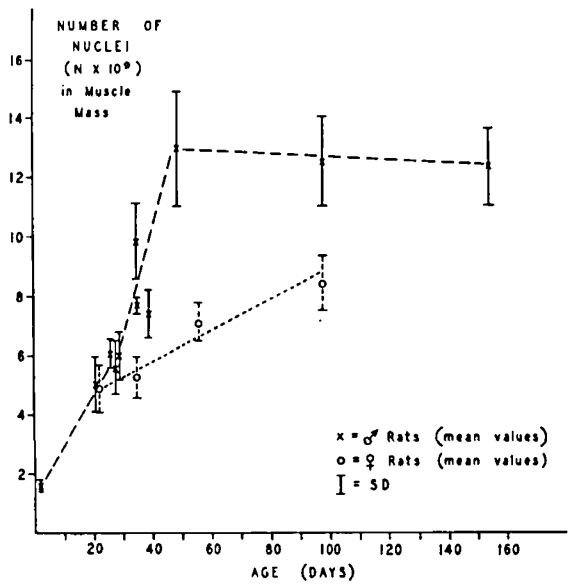

Fig. 4. The number of nuclei in muscle mass for male and female rats is plotted against age. Note the greater increase in the number of nuclei for the male up to sexual maturity, after which the "steady state of cell population" is reached. The female has fewer nuclei in the muscle than does the male. The standard deviations are shown.

Table III. Macaca mulatta—Normal (N = 5) 2.5 years

	Muscle[1]				
	Biceps	Pectoralis	Psoas	Quadriceps	Gastrocnemius
DNA, mg/g	0.960[2] ± 0.113[3]	0.939 ± 0.128	0.939 ± 0.146	0.958 ± 0.067	0.983 ± 0.128
Protein, mg/g	223.5 ± 11.2	220.6 ± 9.8	221.1 ± 11.2	224.5 ± 9.2	234.7 ± 4.7
Water, mg/g	775.7 ± 8.3	782.4 ± 11.9	781.0 ± 10.5	782.4 ± 13.8	779.1 ± 12.4
Protein/DNA	235.6 ± 32.4	239.4 ± 40.7	240.4 ± 40.4	235.2 ± 19.7	241.8 ± 29.6

[1] Significance:

	Protein	H_2O
Pectoralis *vs* gastrocnemius	P = 0.02	P = 0.025
Psoas *vs* gastrocnemius	P = 0.02	P = 0.02

[2] Mean

[3] SD.

ious mammals ranging in size from the mouse to the horse. All were young adult males. The mass of cytoplasm per nucleus in muscle increased twofold from small to large animals. The horse (posterior thigh muscle) had twice the amount of protein/DNA by comparison with the mouse, but RNA/DNA remained constant. By contrast in the liver RNA/DNA decreased with increasing animal size, as does protein turnover and metabolic rate.

Table III shows protein and DNA concentrations present in various muscle groups of five male monkeys (*Macaca mulatta*) at 2.5 years of age. Although agreement exists between muscle groups for protein/DNA ratios, concentrations of protein and water varied significantly (P = 0.025) between the gastrocnemius and the psoas and pectoralis muscles. The gastrocnemius muscle would appear to depart slightly from other muscle groups.

Table IV contains information on muscle for adult male monkeys of about 4 years. These primates had been subjected to thoracic spinal lesions and removal of a portion of frontal cerebral cortex and some atrophy had occurred in the lower limb muscles. Possibly nerve roots from the lower cervical region were involved. The gastrocnemius muscle shows the highest DNA concentration while the values for protein appear reduced. Other muscle groups also show slightly reduced protein concentrations (Table III) while DNA concentrations are definitely less. With aging, the DNA concentration in muscle declines. Exact ages of the adult monkeys are not known, but a loss of cytoplasm relative to DNA is suspected for the gastrocnemius muscle. The key point is that under most circumstances a muscle sample is probably representative of muscle as a whole; however, muscle atrophy due to central nervous system involvement is an exception.

The DNA unit in human muscle. In the study of muscle growth in the human [140] small samples of the gluteal muscle were taken at biopsy from children over 2 years of age while at earlier ages samples were taken from the abdominal rectus muscle in infant males at the time of hernia repair [124]. The samples were analyzed for protein, collagen, chloride, water, and DNA as described elsewhere [15d]. It has been assumed that muscle samples reflect the overall growth of muscle and that creatinine excretion over a 3-day period, in the presence of a low creatinine diet, reflects muscle mass.

Figure 5 illustrates the changes of protein/DNA with growth. Quadratic equations are described for each sex, and the data are plotted against muscle mass. Once again, the accelerated growth of muscle cell size for the female can be distinguished. The curves for the male and female, however, show only a tenuous statistical difference. The relation for the male can be expressed satisfactorily by a linear equation, but that for females can be expressed only by a quadratic equation.

Table IV. Macaca mulatta with spinal and cerebral (frontal cortex) lesions[1]

	Muscle			
	Biceps	Pectoralis major	Gastrocnemius	Deltoid
DNA[2], mg/g	0.654[3] ± 0.130[4]	0.706 ± 0.130	0.926 ± 0.317	0.679 ± 0.155
Protein, mg/g	217.9 ± 18.1	218.4 ± 14.9	212.4 ± 14.2	210.2 ± 16.3

[1] Nine adult animals, about 4 years of age.

[2] DNA: biceps *vs* pectoralis major $P < 0.01$; biceps *vs* gastrocnemius $P < 0.02$; pectoralis major *vs* gastrocnemius $P < 0.025$; deltoid *vs* gastrocnemius $P < 0.025$.

[3] Mean

[4] SD.

The DNA value for the gastrocnemius muscle is higher than for other muscle groups, and consequently the protein/DNA ratio is reduced, indicating some degeneration in this muscle at this age.

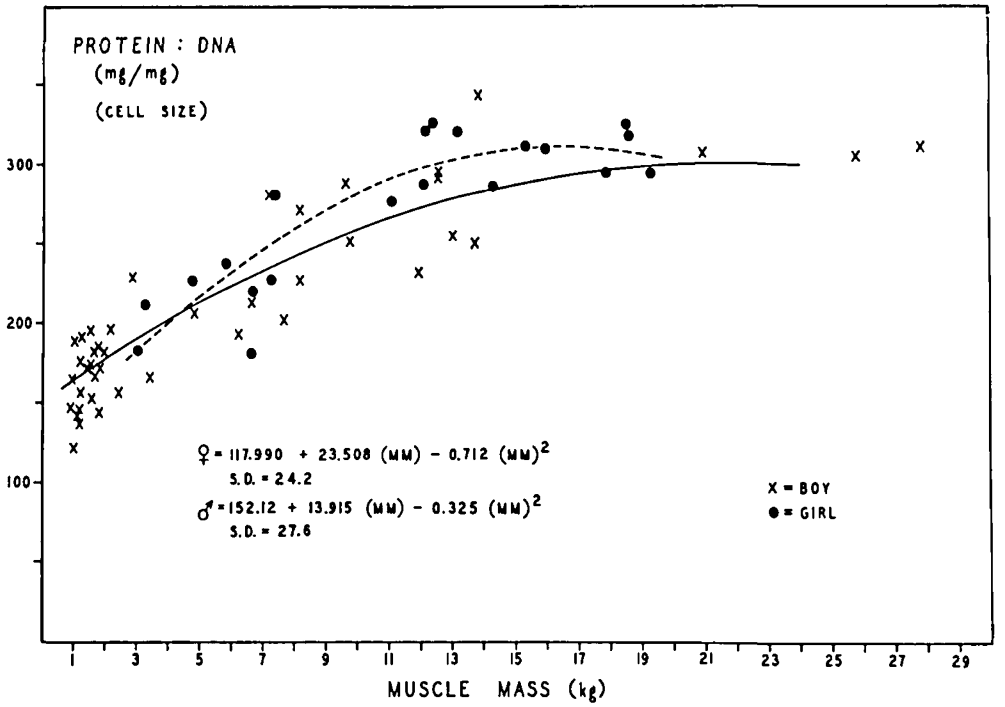

Fig. 5. The protein/DNA ratio in muscle for the human up to 17 years is plotted against the muscle mass (from creatinine excretion). Note that, as for the rat, the female has larger muscle cells but eventually the male catches up with respect to this dimension and possibly the human male could show a further spurt in protein/DNA after 17 years (as for the rat). It is noteworthy that increases in protein/DNA are minimal during growth for the human as compared with the rat. (Reprinted from Fed. Proc., *29:* 1503 (1970).)

If age is placed on the abscissa, the scatter of points is more remarkable, but the female shows larger protein/DNA ratios at an earlier age. This observation pertaining to the female has been confirmed histologically [62]. The male eventually possesses a muscle cell size equal to that of the female. Similarities with muscle growth in the rat are clear, but the present data suggest that the increments in cell size are only two-fold for the human. There is no evidence that the relation is cubic for the human male. No investigations have been carried out for males 17–25 years of age. As to whether the ratio increases further in the human male remains indeterminate at present.

The ability to predict the number of nuclei in the musculature of humans depends on a correct measurement of muscle mass and on the muscle sample being representative of muscle tissue in general.

Figure 6 shows the changes in the number of nuclei within the musculature of children and adolescents when chronological age is the base line. For boys, a cubic equation is defined and a closer relation holds for age than for height [20]. This finding emphasizes the fact that cell replication is time-dependent and cell number is a sensitive index of maturation. Indeed, it is possible to reverse this equation and use cell number to predict biologic or physiologic age for the growth-retarded child [15e].

From the cubic equation for the male, it appears muscle growth is accelerated prior to 2 years and following 9 years of age (adolescent spurt). Clearly, increments in the number of nuclei cannot continue. Figure 6 predicts that a 14-fold increase occurs from infancy to adolescence, but some early maturing boys with nearly completed height growth at 18 years have, we find, a value in muscle of 4×10^{12}, which predicts that a 20-fold increase is possible. Indeed, the 20-fold increase in the DNA content of the muscle mass of the human male is remarkable, and if it is accepted that the majority of this DNA is within the muscle fiber, then one must also accept the thesis that nuclear division is conspicuous. Possibly satellite cells (see *"Introduction"*) play a central and not a secondary role in muscle growth.

Girls from 3 to 17 years have a less remarkable increase in muscle DNA content; the relation can be expressed as a simple linear equation. If the pattern of growth is similar for infant boys and girls, the mathematical expression would be quadratic for girls. It

would appear that girls at 17 years have only two-thirds the number of nuclei in muscle as boys. There is a similar sex-related difference observed for mature rats. It is clear, however, that muscle growth in humans is far less dependent on cell size increase and more on replication of nuclei, while in the rat increments in the number of nuclei are less remarkable, but changes in the protein/DNA more prominent.

In Table V, the relation between the number of muscle nuclei and body length is documented; it is quadratic for the male and linear for the female. Sex differences disappear when intracellular water (mass) is on the abscissa, and a linear relation can be described for data from boys and girls.

The relation between protein/DNA of muscle against height is linear for both boys and girls. The individual cell mass for muscle when plotted against creatinine excretion yields a quadratic relation for male infants and boys, but the relation is linear for girls, possibly because there is a lack of data for infant girls.

The DNA unit has functional significance in muscle. Arguments as to whether fiber numbers increase

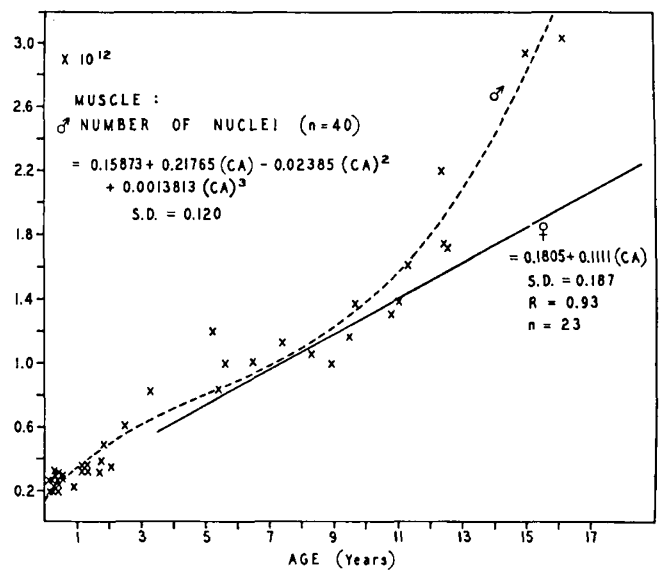

Fig. 6. The number of nuclei in human muscle mass is plotted against age for males (infancy to 17 years) as shown by crosses. For females (4–18 years) points are not shown. The relation is cubic for males and linear for females. Increments for males are more remarkable prior to 2 years and after 9 years (adolescent spurt). The figure shows a 14-fold increase in the number of nuclei for the postnatal male. Additional information would suggest that this value may reach 20. Thus, increments in number of nuclei are more remarkable for the human than for the rat. The sex difference between human males and females is not obvious. (Reprinted from Fed. Proc., *29:* 1503 (1970).)

Table V. Human muscle (additional equations)

Number of nuclei: (NN × 10^{12}) in muscle mass *vs* height (HT) in cm for ♂ (infants and boys up to 18 years) (N = 40)
NN = 1.16727 − 0.0284123 (HT) + 0.000220026 (HT)² SD = 0.172
for ♀ (4–17 years) (N = 22)
NN = −1.60169 + 0.02153 (HT)
where r = 0.924 and SD = 0.183
Number of nuclei: (NN × 10^{12}) in muscle mass *vs* intracellular water (ICW) in liters for children 4–17 years.
♂ + ♀ (no sex difference) (N = 41)
NN = −0.0801 + 0.1240 (ICW)
where r = 0.912 and SD = 0.263
Individual cell mass (ICM) picograms, *vs* creatinine excretion (CREAT) in mg/24 hr
for ♂ (infants and boys up to 18 years) (N = 36)
ICM = 2939.8901 + 9.4513 (CREAT)
− 0.00493974 (CREAT)²
where SD = 934
for ♀ (4–17 years) (N = 19)
ICM = 4987.99 + 3.145 (CREAT)
where r = 0.838 and SD = 549
Protein/DNA (PD) mg/mg in muscle *vs* height (HT) in cm
♂ (infants and boys to 17 years) (N = 42)
PD = 78.3942 + 1.3312 (HT)
where r = 0.87 and SD = 28.1
♀ (4–17 years) (N = 19)
PD = −2.504 + 2.074 (HT)
where r = 0.86 and SD = 25.9

with growth can be avoided by viewing the cytoplasmic nuclear ratio as a discrete and fundamental cell unit within the muscle fiber, as is the case for myocardial tissue [139]. Such an approach is more physiological, or functional, than anatomical since the concept assumes each nucleus has jurisdiction over a finite volume of cytoplasm. With growth, the mechanisms for DNA replication and protein accretion are set into motion.

Factors Influencing Muscle Growth

Exercise. Malina [86] has recently reviewed the subject of vigorous and sustained exercise leading to muscle hypertrophy, with inactivity causing the reverse. Christensen and Crampton [31] have shown in 1-year-old rats that forced exercise during the year causes an increase in DNA, RNA, and protein in the gastrocnemius muscles. From their data, however, it seems there is no increase in protein/DNA ratio between exercised rats and a control group. Thus, it is suggested that exercise causes muscle growth and increments in protein and DNA are commensurate.

Morpurgo [98] originally considered muscular hypertrophy to be due to an increase in sarcoplasm, while

Holmes and Rasch [68] found no increase in the number of myofibrils within the myofiber. Helander [64] confined guinea pigs and rabbits for 3 years. There was reduced myofilamental but enhanced sarcoplasmic content. Helander concluded that skeletal muscle adapts itself to functional activity with hypertrophy after some types of exercise, and atrophy after restriction in activity [53]. Goldberg (Endocrinology *83:* 1071, 1968) has shown that acute work hypertrophy in the soleus or plantaris muscle of rats (following section of the gastrocnemius muscle) can be induced in hypophysectomized or alloxan-diabetic rats, thus suggesting that neither growth hormone nor insulin is necessary for that process. No information is available on DNA or protein content of these muscles.

Hormones. The value of inspecting protein/DNA ratio in muscle and the number of nuclei within muscle mass can be illustrated further by considering certain abnormal situations. Studies on rats hypophysectomized at 21 days revealed at 35, 38, 49, and 56 days of age that there was no increment in the DNA content of muscle with time [18, 23]. When tissue studies were carried out at 6 or 8 weeks of age, similar findings occurred in rats given ^{131}I at 1 week of age to ablate the thyroid [23]. Since thyroid insufficiency in rats leads to secondary degeneration of acidophil cells [110, 120], it was suggested earlier [16, 23] that growth hormone is important for DNA replication in muscle. That such is the case [3, 18] is illustrated in Figure 7 (left side). This figure defines the changes in muscle when hypophysectomized rats are treated for 11 days with (*a*) bovine growth hormone, (*b*) protamine zinc insulin, or (*c*) growth hormone with reduced endogenous insulin [18]. The endogenous insulin was blocked by injection of epinephrine [141] [109]. Rats injected with insulin or growth hormone received a food intake comparable to that given untreated hypophysectomized rats. In *groups a* and *c,* DNA increased significantly while the injection of insulin caused only a minimal increase. The DNA increase in *groups a* and *c* was no doubt due to gain in numbers of nuclei within the muscle fiber, while the lesser gain in *group b* could more readily be ascribed to increments in the number of nuclei in connective tissue. Insulin causes growth of collagen in absence of the pituitary gland [18] and has an effect on DNA replication in adipose tissue [43], especially in the stromal tissue [67].

Figure 8 shows that hypophysectomized rats have an increased protein/DNA ratio relative to body size.

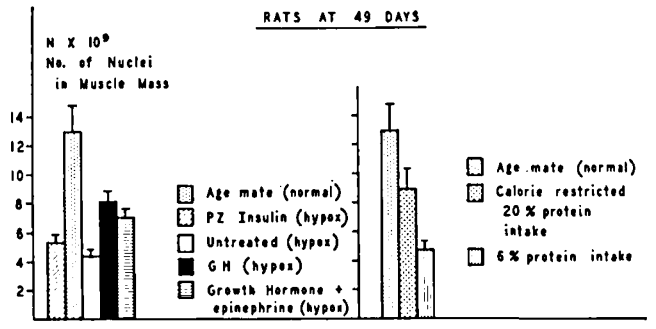

Fig. 7. Left side: Increments in number of nuclei in muscle of rats hypophysectomized at 21 days when treated from the 38th to 49th day with various hormones. Note that bovine growth hormone with ($P < 0.005$) or without ($P < 0.001$) the blocking of endogenous insulin (using epinephrine) causes large increments in the number of nuclei. Exogenous insulin produces lesser effects ($P < 0.005$). One standard deviation is shown for each column. *Right side:* Similar data are shown for rats receiving restricted calorie intake (with normal protein intake) and for rats receiving low protein and low calorie intake from 23rd to 49th day. Note that restriction of calories produces some reduction from the expected number of nuclei (33%) ($P < 0.001$), while restriction of both components produces gross reduction ($P < 0.001$) or values almost equivalent to those present in a 23-day-old rat. Of interest is the fact that the protein/DNA increases in calorie restriction but decreases in protein plus calorie restriction (not shown).

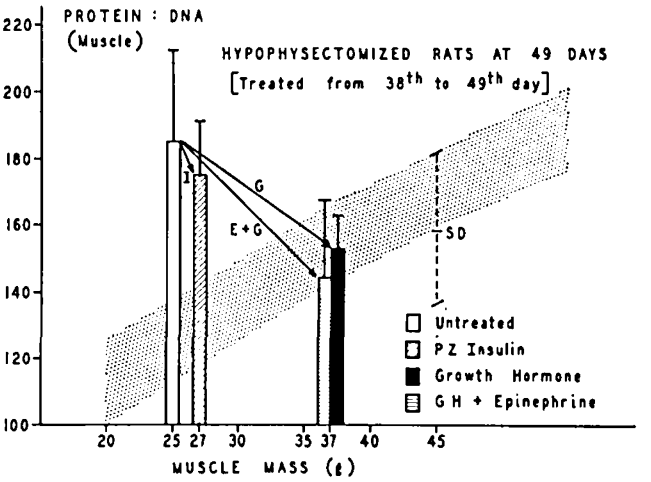

Fig. 8. Hypophysectomized rats (referred to in Fig. 7) have been treated with various hormones and the protein/DNA of muscle plotted against the muscle mass. Note that the hypophysectomized rat has a large protein/DNA relative to organ size (muscle mass) ($P < 0.001$). The introduction of growth hormone with or without the blocking of endogenous insulin diminished the ratio to normal while exogenous insulin had no significant effect. The protein/DNA remained excessive ($P < 0.001$). Indeed, the question arises as to whether the large protein DNA ratio of the untreated hypophysectomized rat is related to the unopposed action of endogenous insulin. One standard deviation is shown for each column. The arrows from the column representing the untreated hypophysectomized rat illustrate hormonal treatments. I: PZ insulin. G: Growth hormone. E + G: Growth hormone plus epinephrine.

With bovine growth hormone, with or without the blocking of endogenous insulin, the ratio is restored to normal. On the other hand, insulin does not reduce the existing high protein/DNA ratio. This hormone is likely to be involved in cytoplasmic growth. Indeed, the fact that hypophysectomized rats have a significant increase in the protein/DNA ratio may be due to the fact that endogenous insulin predominates in the absence of growth hormone. The intact rat, if given insulin for the same period of time, shows growth of muscle mass, which is due to increase in the protein/DNA ratio [58].

In the human, growth hormone also increases the DNA of the muscle mass. Patients with idiopathic pituitary insufficiency [16] have a reduction in the number of nuclei in muscle. Catch-up growth following treatment with human growth hormone is associated with a spurt in the number of nuclei. In fact, muscle growth outstrips skeletal growth [15f]. No consistent change could be found in muscle cell size, neither in protein/DNA ratio nor in individual cell mass.

Mice with hereditary pituitary insufficiency yield findings in muscle similar to those in children with hypopituitarism. Snell-Smith dwarf mice have a reduction in the number of nuclei present without an increase in cell size relative to body size. In pituitary insufficiency, multiple hormonal factors may be missing so that influences on protein/DNA are difficult to delineate.

Children with acquired hypothyroidism have a less than normal number of nuclei in muscle as well as a distinctly high protein/DNA ratio. These patients show changes in muscle similar to those found in rats hypophysectomized at weaning. In acquired hypothyroidism, there is a poor release of growth hormone [11, 45, 71, 84]. Since thyroid hormone is important for proper action of growth hormone [54], it can be speculated that insulin may be exerting, in this disease, a stronger influence on muscle growth. Treatment with thyroid hormone increases the number of nuclei in muscle while cell size returns toward normal. The same result is seen in the hypophysectomized rat given growth hormone. Patients with congenital hypothyroidism have a reduction in protein/DNA ratio in muscle [16]. Thyroid hormone appears to have a greater action on protein synthesis earlier in life than later [119], which could explain to some extent the differential effects on cell size of muscle in congenital *versus* acquired hypothyroidism.

Insulin is possibly the major hormone responsible for protein synthesis after infancy. It is clear, however, that androgens also stimulate muscle cell growth at puberty, especially protein synthesis. The role of androgens in muscle growth has been reviewed by Kochakian [77]. Our own work on adrenalectomized castrated male rats did not show, however, great changes in the growth of muscle mass, DNA, or protein content [15b].

Nutrition. Nutrition has a profound effect on muscle growth [53]. In rats, calorie restriction in the postweaning period (3–7 weeks) with a borderline protein intake causes reduction in DNA content (compared with age mates) with an increase in the protein/DNA or RNA/DNA ratios for age, while feeding *ad libitum* subsequently provides remarkable "catch-up" growth [15i, 40, 58]. Hill *et al.* [65] noticed that feeding adequate protein with reduced calories did not cause a loss of cell size but did result in the finding of less DNA in the muscle mass (66% of controls). The findings of Durand *et al.* [38] were similar. Moreover, they found that eventually such rats attain a full complement of nuclei within the muscle. Thus, restriction of calories (with normal amounts of protein) slows cell number increase during growth.

By contrast, Winick and Noble [134], by restricting food intake to 50% (calorie and protein restriction) in postweanling rats, produced permanent growth retardation with reduction of expected cell number. In 1958, Mendes and Waterlow [92] demonstrated grossly reduced protein/DNA ratios in muscle of protein-deficient postweanling rats, while the work of Young and Alexis [138] showed that ribosomal activity was compromised. Of importance is the realization that rats voluntarily restrict their calorie intake when presented with a low protein diet. Clearly the evidence at hand suggests that there is a difference between calorie restriction and calorie plus protein restriction with respect to muscle cell growth of rats.

Hill *et al.* [65] showed that with protein plus calorie restriction (3–7 weeks) the plasma insulin levels were significantly low in rats together with reduced protein/DNA ratios, while the DNA content in muscle was only 33% of that found in controls (Figure 7, right side). On the available evidence it is suspected that protein intake is mainly related to cytoplasmic growth, possibly through the action of insulin, while caloric intake would appear to influence the multiplication of muscle nuclei, possibly through growth hormone. Separation of these factors may be very important as the implication is that only protein restriction in the postweaning period causes permanent growth retardation.

In boys a quadratic relation exists between calorie intake and age [14]. A similar relation also holds for the number of nuclei present in the muscle mass at

various ages, and it can be anticipated that a linear relation exists between number of nuclei and caloric intake. As with rats, significant changes are found in human muscle in patients subjected to restricted nutrition. The study of Peruvian infants with kwashiorkor or marasmus (protein-calorie malnutrition) reveals that there is a gross reduction in the protein/DNA ratio in muscle for body size as well as a reduction in RNA/DNA. Rehabilitation did not restore levels to those expected for Caucasian infants [21]. Histological observations of muscle by Montgomery [96] confirmed the gross reduction of fiber size, while nuclei appeared overabundant. The reduction in creatinine excretion was found to be proportional to the reduction of the total intracellular mass in our patients [21], suggesting that creatinine excretion still reflected muscle mass. No great reduction in the number of nuclei in the muscle was found in these infants shortly after beginning therapy or after rehabilitation. The finding again raises the question as to whether muscle growth in the primate is different from that in rodents, and whether the permanent reduction of nuclei in muscle and other tissues, which eventually occurs in rats subject to protein restriction early in postnatal life [134], does in fact occur in the human. On a DNA basis, protein and RNA in muscle do not return to normal status in rehabilitated infants; this suggests that some permanent damage may exist with respect to protein-synthesizing mechanisms. Moreover, insulin levels in the plasma were low, and, even after rehabilitation, the infusion of arginine did not produce a satisfactory response [56]. Once again, a relation can be drawn between protein intake, cell size, and insulin release.

The study of overnutrition and obesity in female adolescents [25] revealed, as in experimental animals [44, 76, 133, 135], that an increase in the number of nuclei and overgrowth of lean tissues occurs. Obese girls with advanced bone age all had increases in muscle mass and DNA content within the muscle mass when body length was used as the base line. The protein/DNA ratio was diminished [25]. An accelerated increase of protein/DNA ratio in muscle is characteristic of the female while increments in the number of nuclei progress at a slower rate. In female rats, this situation can be countered by castration. Clearly, the question arises as to whether estrogen secretion is suppressed, or androgen secretion augmented, in some obese females. Their pattern of growth in muscle is characteristic of excessive response to growth hormone with diminished response to insulin. Hansen [61] has shown that nonobese diabetics secrete excessive amounts of growth hormone when under exercise and when not properly controlled with insulin. In view of the link between diabetes and obesity, one wonders whether obese patients might not have abnormal secretion of growth hormone. It is known that certain tissues in obese patients become resistant to circulating insulin [113]. In this direction it is interesting to point out that Stauffacher *et al.* (Ann N. Y. Acad. Sci., *131:* 374, 1965) demonstrated that the sensitivity of muscle to insulin in mice is much more inhibited than the sensitivity of adipose tissue.

Much more work is required to evaluate changes in muscle growth with respect to hormones and nutrition. It is clear at present that hormones, in addition to androgens, profoundly affect muscle growth, and that valuable information on growth can be obtained from the appraisal of muscle mass, the inspection of DNA content, and the ratio of protein/DNA, or the appraisal of the mass of individual cells. A better understanding of growth retardation and obesity may be reached if the implication is accepted that caloric intake is important to the rate of increase of muscle nuclei.

Conclusions

Histometric approaches to muscle growth have produced limited quantitative information, while measurement of protein and DNA in muscle tissue and of their ratio gives a functional or physiological unit that is meaningful. Admittedly, the chemical approach does not define what percentage of nuclei actually belong to the muscle fiber. Current work indicates that one-quarter of the DNA in a muscle sample resides outside the muscle fiber. Consideration of the number of nuclei in muscle and the extent of protein accretion is compatible with physiological concepts and the discrete actions of hormones. A chemical approach, if combined with a histological approach designed to differentiate cell types, should yield valuable information. Perhaps the previous lack of appreciation of the above discussion has rested on the old belief that there was little increase in the number of nuclei in muscle during postnatal life. Indeed, even now with the discovery of the satellite cell by Mauro, the role, or potential, of this cell is still not anticipated fully, and concern is expressed mainly at its potential with respect to muscle injury, rather than toward growth.

While certain types of exercise cause increments in muscle growth, it is of interest that the protein/DNA ratio does not change. Disproportionate changes are

revealed when hormonal and nutritional factors alter, all of which supports the concept that the nucleus dominates a finite volume of cytoplasm, the DNA unit, and each component can be modified by separate factors.

If proper account is to be taken of abnormal growth in the human, it is important to account for changes in the number of nuclei within the muscle mass in cytoplasm relative to the nucleus. The measurement of muscle mass by studies of creatinine excretion, under properly controlled circumstances, or the study of the distribution of labeled creatine (with ^{15}N or ^{14}C) provide acceptable methods of approach. Both in rats and primates, a muscle sample from a muscle group would appear to be representative of the entire musculature, so that the prediction of the number of nuclei is possible from data concerning the composition of the sample and total muscle mass.

The value of assessing normal and abnormal growth clinically by inspection of the size and number of cells was pointed out in 1964 [17], and work in the intervening years has only reemphasized the thinking. The relation of cell growth to nutrition and hormones is fundamental to the understanding of fetal growth, postnatal growth, and, eventually, the process of aging with negative growth. The fact that skeletal muscle represents more than two-thirds of metabolically active protoplasm and is readily accessible for study makes this tissue one of great importance for future research into human development.

References and Notes

1. Albanese, A. A., and Orto, L.: Protein and amino acids. In: A. A. Albanese: Newer Methods of Nutritional Biochemistry, Vol. 3, p. 1 (Academic Press, New York, 1967).
2. Bardeen, C. R.: Development of musculature of the body wall in the pig. Johns Hopkins Hosp. Rep., *9:* 36 (1900).
3. Beach, R. K., and Kostoyo, J. L.: Effect of growth hormone on the DNA content of muscles of young hypophysectomized rats. Endocrinology, *82:* 882 (1968).
4. Bintliff, S., and Walker, B. E.: Radioautographic study of skeletal muscle regeneration. Amer. J. Anat., *106:* 233 (1960).
5. Bischoff, R., and Holtzer, H.: Mitosis and the processes of differentiation of myogenic cells in vitro. J. Cell. Biol., *41:* 188 (1969).
6. Bloch, K., and Schoenheimer, R.: Studies in protein metabolism. XI. The metabolic relation of creatine and creatinine studied with isotopic nitrogen. J. Biol. Chem., *131:* 111 (1939).
7. Boivin, A., Vendreley, R., and Vendreley, C.: L'acid desoxyribonucleique du noyau cellulaire depositaire des caracteres hereditaires: arguments d'ordre analytique. C. R. Acad. Sci., *226:* 1061 (1948).
8. Borsook, H., and Dubnoff, J. W.: The hydrolysis of phosphocreatine and the origin of urinary creatinine. J. Biol. Chem., *168:* 493 (1947).
9. Bowden, D. H., and Goyer, R. A.: The size of muscle fibers in infants and children. A.M.A. Arch. Pathol., *69:* 188 (1960).
10. Boyd, J. D.: Development of striated muscle. In: G. H. Bourne: The Structure and Function of Muscle, p. 63 (Academic Press, New York, 1960).
11. Brauman, H., and Corvilain, J.: Growth hormone response to hypoglycemia in myxoedema. J. Clin. Endocrinol., *28:* 301 (1968).
12. Butterfield, R. M.: Prediction of muscle content of steer carcasses. Nature, *195:* 193 (1962).
13. Caster, W. O., Poncelet, J., Simon, A. B., and Armstrong, W. D.: Tissue weights of rat; normal values determined by dissection and chemical methods. Proc. Soc. Exp. Biol. Med., *91:* 122 (1956).
14. Cheek, D. B.: Cellular growth, hormones, nutrition and time. Borden Award Address, October, 1967. Pediatrics, *41:* 30 (1968).
15. Cheek, D. B. (Editor): Human Growth: Body Composition, Cell Growth, Energy, and Intelligence, pp. (a) 150, (b) 306, (c) 16, (d) 653, (e) 541, (f) 620, (g) 463, (h) 117, (i) 326 (Lea and Febiger, Philadelphia, 1968).
16. Cheek, D. B., Brasel, J. A., Elliott, D., and Scott, R. E.: Muscle cell size and number in normal children and in dwarfs (pituitary, cretins and primordial) before and after treatment. Bull. Johns Hopkins Hosp., *119:* 46 (1966).
17. Cheek, D. B., and Cooke, R. E.: Growth and growth retardation. Annu. Rev. Med., *15:* 357 (1964).
18. Cheek, D. B., and Graystone, J. E.: The action of insulin, growth hormone and epinephrine on cell growth in liver, muscle and cerebrum of the hypophysectomized rat. Pediat. Res., *3:* 77 (1969).
19. Cheek, D. B., Graystone, J. E., and Mehrizi, A.: The importance of muscle cell number in children with congenital heart disease. Bull. Johns Hopkins Hosp., *118:* 140 (1966).
20. Cheek, D. B., and Hill, D. E.: Muscle and liver cell growth: Role of hormones and nutritional factors (calories, protein, Zn). Fed. Proc., *29:* 1503 (1970).
21. Cheek, D. B., Hill, D. E., Cordano, A., and Graham, G. G.: Malnutrition in infancy: Changes in muscle and adipose tissue before and after rehabilitation. Pediat. Res., *4:* 135 (1970).
22. Cheek, D. B., and Holt, A. B.: Growth and body composition in the mouse. Amer. J. Physiol., *205:* 913 (1963).
23. Cheek, D. B., Powell, G. K., and Scott, R. E.: Growth of muscle cells (size and number) and liver DNA in rats and Snell Smith mice with insufficient pituitary, thyroid or testicular function. Bull. Johns Hopkins Hosp., *117:* 306 (1965).
24. Cheek, D. B., Powell, G. K., and Scott, R. E.: Growth and muscle mass and skeletal collagen in the rat. I. Normal growth. Bull. Johns Hopkins Hosp., *116:* 378 (1965).
25. Cheek, D. B., Schultz, R. B., Parra, A., and Reba, R. D.: The overgrowth of lean and adipose tissues in adolescent obesity. Pediat. Res., *4:* 268 (1970).
26. Cheek, D. B., and West, C. D.: An appraisal of methods of tissue chloride analysis: The total carcass chloride, ex-

changeable chloride, potassium and water of the rat. J. Clin. Invest., *34:* 1744 (1955).
27. CHEEK, D. B., AND WEST, C. D.: Alterations in body composition with sodium loading and potassium restriction in the rat: the total body sodium, nitrogen, magnesium and calcium. J. Clin. Invest., *35:* 763 (1956).
28. CHEEK, D. B., WEST, C. D., AND GOLDEN, C. C.: The distribution of Na and Cl and the extracellular fluid volume in the rat. J. Clin. Invest., *36:* 340 (1957).
29. CHIAKULAS, J. J., AND PAULY, J. E.: A study of postnatal growth of skeletal muscle in the rat. Anat. Rec., *152:* 55 (1965).
30. CHINN, K. S. K.: Potassium and creatinine as indexes of muscle and nonmuscle protein in rats. J. Nutr., *90:* 323 (1966).
31. CHRISTENSEN, D. A., AND CRAMPTON, E. W.: Effects of exercise and diet on nitrogenous constituents in several tissues of adult rats. J. Nutr., *86:* 369 (1965).
32. CHURCH, J. C. T., NORONHA, R. F., AND ALLBROOK, D. B.: Satellite cells and skeletal muscle regeneration. Brit. J. Surg., *53:* 638 (1966).
33. CLARK, L. C., THOMPSON, H. L., BECK, E. I., AND JACOBSON, W.: Excretion of creatinine and creatine by children. Amer. J. Dis. Child., *81:* 774 (1951).
34. COUTEAUX, R.: Recherches sur l'histogenese du muscle strie des mammiferes et al formation des plaques motrices. Bull. Biol. Fr. Belg., *75:* 101 (1941).
35. DEUEL, H. J., JR., SANDIFORD, I., SANDIFORD, K., AND BOOTHBY, W. B.: A study of nitrogen minimum: The effect of sixty-three days of protein free diet on the nitrogen partition products in the urine and on the heat production. J. Biol. Chem., *76:* 391 (1928).
36. DUBOWITZ, V.: Developing and diseased muscle: A histochemical study S.I.M.P. Res. Monog. no. 2, p. 1 (1968).
37. DUNNILL, M. S.: In: S. C. Dyke: Quantitative Methods in Histology, Recent Advances in Clinical Pathology, Ser. V, p. 401 (Little, Brown and Company, Boston, 1968).
38. DURAND, G., FAUCONNEAU, G., AND PENOT, E.: Croissance des tissu du rat et reduction de l'apport energetique de la ration; influence sur la teneur en acides nucleiques. Ann. Biol. Anim. Biochim. Biophys., *7:* 145 (1967).
39. ELLIOTT, D. A., AND CHEEK, D. B.: Muscle electrolyte patterns during growth. In: D. B. Cheek: Human Growth, p. 260 (Lea and Febiger, Philadelphia, 1968).
40. ELLIOTT, D. A., AND CHEEK, D. B.: Muscle cell growth in rats with exposure to hypoxia and food restriction. J. Pediat., *69:* 958 (1966).
41. ENESCO, M., AND PUDDY, D.: Increase in the number of nuclei and weights in skeletal muscle of rats of various ages. Amer. J. Anat., *114:* 235 (1964).
42. EPSTEIN, C. J.: Cell size, nuclear content and the development of polyploidy in the mammalian liver. Proc. Nat. Acad. Sci. U.S.A., *57:* 327 (1967).
43. ESANU, C., MURAKAWA, S., BRAY, G. A., AND RABEN, M. S.: DNA synthesis in human adipose tissue in vitro. I. Effect of serum and hormones. J. Clin. Endocrinol. Metabol., *29:* 1027 (1969).
44. FILER, L. J., AND CHURELLA, H.: Relationship of body composition, chemical maturation, homeostasis and diet in the newborn mammal. Ann. N.Y. Acad. Sci., *110:* 380 (1963).
45. FINK, C. W.: Thyrotropin deficiency in a child resulting in secondary growth hormone deficiency. Pediatrics, *40:* 881 (1967).
46. FOLIN, O.: Laws governing the chemical composition of urine. Amer. J. Physiol., *13:* 66 (1905).
47. FORBES, R. M., MITCHELL, H. H., AND COOPER, A. R.: Further studies on the gross composition and mineral elements of the adult human body. J. Biol. Chem., *223:* 969 (1956).
48. GARN, S.: Human biology and research in body composition. Ann. N.Y. Acad. Sci., *110:* 429 (1963).
49. GARN, S., AND CLARK, L. E.: Creatinine-weight coefficient as a measurement of obesity. J. Appl. Physiol., *8:* 135 (1955).
50. GODLEWSKI, E., JR.: Die Entwicklung des Skelet-und-Hertzmuskelgewebes der Saugethiere. Arch. Mikrobiol. Anat., *60:* 111 (1902).
51. GOLDSPINK, G.: Studies on postembryonic growth and development of skeletal muscles. Roy. Irish Acad. Proc., *61:* 135 (1962).
52. GOLDSPINK, G.: Increase in length of skeletal muscle during normal growth. Nature, *201:* 1095 (1964).
53. GOLDSPINK, G.: The combined effects of exercise and reduced food intake on skeletal muscle fibers. J. Cell. Comp. Physiol., *63:* 209 (1964).
54. GOMEZ, C. J., GHITTONI, N. E., AND DELLACHA, J. M.: Effect of L-thyroxine or somatotrophin on body growth and cerebral development in neonatally thyroidectomized rats. Life Sci., *5:* 243 (1966).
55. GORDON, E. E., KOWALSKI, K., AND FRITTS, M.: Muscle proteins and DNA in rat quadriceps during growth. Amer. J. Physiol., *210:* 1033 (1966).
56. GRAHAM, G. G., CORDANO, A., BLIZZARD, R. M., AND CHEEK, D. B.: Infantile malnutrition: Changes in body composition during rehabilitation. Pediat. Res., *3:* 579 (1969).
57. GRAYSTONE, J. E.: Creatinine excretion during growth. In: D. B. Cheek: Human Growth, p. 182 (Lea and Febiger, Philadelphia, 1968).
58. GRAYSTONE, J., AND CHEEK, D. B.: The effects of reduced calorie intake and increased calorie intake (insulin induced) on the cell growth of muscle, liver, and cerebrum, and on skeletal collagen in the post weaning rat. Pediat. Res., *3:* 66 (1969).
59. HALBAN, J.: Die Dicke der quergestreiften Muskelfasern und ihre Bedeutung. Anat. Hefte, *3:* 267 (1894).
60. HAMMOND, J., AND APPLETON, A. P.: Growth and Development of Mutton Qualities in Sheep. (Oliver and Boyd, London, 1932).
61. HANSEN, A. P.: Abnormal serum growth hormone response to exercise in juvenile diabetes. J. Clin. Invest., *49:* 1467 (1970).
62. HARTMANN, W. H.: Histologic study of muscle cell size. In: D. B. Cheek: Human Growth, p. 372 (Lea and Febiger, Philadelphia, 1968).
63. HEIDENREICH, C. H.: Der Einfluss der histologischen bestandeile des muskels auf die qualitat del Fleisches. Wiss. Arch. Landwirtsch, *6:* 366 (1931).
64. HELANDER, E. A. S.: Influence of exercise and restricted activity on the protein composition of skeletal muscle. Biochem. J., *78:* 478 (1961).
65. HILL, D. E., HOLT, A. B., PARRA, A., AND CHEEK, D. B.: The influence of protein or calorie deficiency on the body

composition and cellular growth of muscle and liver in weanling rats. Johns Hopkins Med. J., *127:* 146 (1970).
66. Hoagland, C. L.: Studies of altered metabolism in diseases of muscle. Advan. Enzymol., *VI:* 193 (1946).
67. Hollenberg, C. H., Vost, A., and Patten, R. L.: Regulation of adipose mass: Control of fat cell development and lipid content. Recent Progr. Hormone Res., *26:* 463 (1970).
68. Holmes, R., and Rasch, P. J.: Effect of exercise on number of myofibrils per fiber in sartorius muscle of the rat. Amer. J. Physiol., *195:* 50 (1958).
69. Huxley, H. E.: Evidence for continuity between the central elements of the triads and extracellular space in frog sartorius muscle. Nature, *202:* 1067 (1964).
70. Ishikawa, H.: Electron microscopic observations of satellite cells with special reference to the development of mammalian skeletal muscle. Z. Anat. Entwicklungsgesch., *125:* 43 (1966).
71. Iwatsubo, H., Omori, K., Okada, Y., Fuchuchi, M., Miyai, K., Abe, H., and Kumahara, Y.: Human growth hormone secretion in primary hypothyroidism before and after treatment. J. Clin. Endocrinol., *27:* 1751 (1967).
72. Jackson, C. M., and Lowrey, L. G.: On the relative growth of the component parts (head, trunk and extremities) and systems (skin, skeleton, musculature and viscera) of the albino rat. Anat. Rec., *6:* 449 (1912).
73. Joubert, D. M.: An analysis of factors influencing postnatal growth and development of the muscle fibre. J. Agr. Sci., *47:* 59 (1956).
74. Kelly, A. M., and Zacks, S. I.: The histogenesis of rat intercostal muscle. J. Cell. Biol., *42:* 135 (1969).
75. Klinkerfuss, G. H.: An electron microscopic study of myotonic dystrophy. Arch. Neurol., *16:* 181 (1967).
76. Knittle, J. L., and Hirsch, J.: Effects of early nutrition on the development of rat epididymal fat pads: Cellularity and metabolism. J. Clin. Invest., *47:* 2091 (1968).
77. Kochakian, C. D.: Regulation of muscle growth by androgens. In: E. J. Briskey, R. G. Cassens, and J. C. Trautman: The Physiology and Biochemistry of Muscle as a Food, p. 81 (University of Wisconsin Press, Madison, 1966).
78. Kumar, I., Lund, D. G., and Boyne, A. W.: The determination of body composition of living animals: The daily endogenous creatinine excretion as a measure of body composition in rats. Brit. J. Nutr., *13:* 320 (1959).
79. Latimer, H. B.: The prenatal growth of the cat. XV. The weight of the musculature in the fetal and in the adult cat. Growth, *8:* 205 (1944).
80. Leblond, C. P.: Classifications of cell populations on the basis of their proliferative behaviour. Nat. Cancer Inst. Monogr., *14:* 119 (1964).
81. Lee, J. C.: Electron microscope observations on myogenic free cells of denervated skeletal muscle. Exp. Neurol., *12:* 123 (1965).
82. MacCallum, J. B.: On the histogenesis of the striated muscle fibre, and the growth of the human sartorius muscle. Johns Hopkins Hosp. Bull., *90–91:* 208 (1898).
83. MacConnachie, H. F., Enesco, M., and Leblond, C. P.: The mode of increase in the number of skeletal muscle nuclei in the postnatal rat. Amer. J. Anat., *114:* 245 (1964).
84. MacGillivray, M. H., Aceto, T., Jr., and Frohman, L. H.: Plasma growth hormone responses and growth retardation of hypothyroidism. Amer. J. Dis. Child., *115:* 273 (1968).
85. Malina, R. M.: Quantification of fat, muscle and bone in man. Clin. Orthop. Related Res., *65:* 9 (1969).
86. Malina, R. M.: Exercise as an influence upon growth: Review and critique of current concepts. Clin. Pediat., *8:* 16 (1969).
87. Martin, A. W., and Fuhrman, F. A.: The relationship between summated tissue respiration and metabolic rate in the mouse and dog. Physiol. Zool., *28:* 18 (1955).
88. Mauro, A.: Satellite cell of skeletal muscle fibres. J. Biophys. Biochem. Cytol., *9:* 493 (1961).
89. McMeekin, C. P.: Growth and development in the pig with special reference to carcass quality characters. J. Agr. Sci., *30:* 276 (1940).
90. McMeekin, C. P.: Growth and development in the pig, with special reference to carcass quality characters. J. Agr. Sci., *31:* 1 (1941).
91. Meador, C. K., Kreisberg, R. A., Friday, J. P., Jr., Bowdoin, B., Coan, P., Armstrong, J., and Hazelrig, J. B.: Muscle mass determination by isotopic dilution of creatine-^{14}C. Metabolism, *17:* 1104 (1968).
92. Mendes, C. B., and Waterlow, J. C.: The effect of a low-protein diet, and of refeeding, on the composition of liver and muscle in the weanling rat. Brit. J. Nutr., *12:* 74 (1958).
93. Meves, F.: Ueber Neubildung quergestreifter Muskelfasern nach Beobachtungen am Huhnerembryo. Anat. Anz., *34:* 161 (1909).
94. Milhorat, A. T.: Creatine and creatinine metabolism and diseases of the neuromuscular system. Res. Nerv. Ment. Dis. Proc., *32:* 400 (1953).
95. Mirsky, A. E., and Ris, H.: Variable and constant components of chromosomes. Nature, *163:* 666 (1949).
96. Montgomery, R. D.: Magnesium metabolism in infantile protein malnutrition. Lancet, *ii:* 74 (1960).
97. Montgomery, R. D.: Growth of human striated muscle. Nature, *195:* 194 (1962).
98. Morpurgo, B.: Ueber Activitats—Hypertrophie der willkurlichen muskeln. Virchow's Arch., *150:* 522 (1897).
99. Morpurgo, B.: Ueber die postembryonale Entwicklung der quergestreiften Muskeln von weissen Ratten. Anat. Anz., *15:* 200 (1898).
100. Moscona, A.: Cytoplasmic granules in myogenic cells. Exp. Cell. Res. *9:* 377 (1955).
101. Munro, H. N.: Evolution of protein metabolism in mammals. In: H. N. Munro and J. R. Allison: Mammalian Protein Metabolism, Vol. 3 (Academic Press, New York, 1969).
102. Munro, H. N., and Gray, J. A. M.: The nucleic acid content of skeletal muscle and liver in mammals of different body size. Comp. Biochem. Physiol., *28:* 897 (1969).
103. Nelson, O. E. (Editor): Comparative Embryology of the Vertebrates (Blakiston Company, Toronto, 1953).
104. Novak, L. P.: Age and sex differences in body density and creatinine excretion of high school children. Ann. N.Y. Acad. Sci., *110:* 545 (1963).
105. Okazaki, K., and Holtzer, H.: An analysis of myogenesis using fluorescein-labeled actomyosin. J. Histochem. Cytochem., *13:* 726 (1965).
106. Orme, L. E., Cole, J. W., Kincaid, C. M., and Cooper, R. J.: Predicting total carcass lean in mature beef from weights of certain entire muscles. J. Anim. Sci., *19:* 726 (1960).
107. Pearson, A. M.: Body composition. In: A. A. Albanese: New

Methods of Nutritional Biochemistry, Vol. 2, p. 1 (Academic Press, New York, 1967).
108. PERRY, S. V., AND ZYDOWO, M.: A ribonucleoprotein of skeletal muscle and its relation to the myofibril. Biochem. J., *72:* 682 (1959).
109. PORTE, D.: A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest., *46:* 86 (1967).
110. PURVES, H. D., AND GREISBACH, W. E.: Observations on the acidophil cell changes in the pituitary in thyroxine deficient states. I. Acidophil degranulation and extra thyroidal thyroxine synthesis. Brit. J. Pathol., *27:* 170 (1946).
111. REZNIK, M.: Possible origin of myoblasts during muscle regeneration. J. Cell. Biol., *39:* 110 (1968).
112. REZNIK, M.: Thymidine-^{3}H uptake by satellite cells of regenerating skeletal muscle. J. Cell. Biol., *40:* 568 (1969).
113. SALANS, L. B., KNITTLE, J. L., AND HIRSCH, J.: The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. J. Clin. Invest., *47:* 153 (1968).
114. SCHIEFFERDECKER, P.: Muskeln und Muskelkern. (Barth, Leipzig, 1909).
115. SHAFFER, P. A.: The excretion of kreatinin and kreatin in health and disease. Amer. J. Physiol., *23:* 1 (1908).
116. SHAFIQ, S. A., GORYCKI, M. A., AND MAURO, A.: Mitosis during postnatal growth in skeletal and cardiac muscle of the rat. J. Anat., *103:* 135 (1968).
117. SIEBERT, W. W.: Untersuchungen uber Hypertrophie des Skeletmuskels. Z. Klin. Med., *109:* 350 (1928).
118. SLAUTTERBACK, D. B.: The ultrastructure of cardiac and skeletal muscle. In: E. J. Briskey, R. G. Cassen, and J. C. Trautman: The Physiology and Biochemistry of Muscle as a Food, p. 39 (University of Wisconsin Press, Madison, 1966).
119. SOKOLOFF, L.: Action of thyroid hormones and cerebral development. Amer. J. Dis. Child., *111:* 498 (1967).
120. SOLOMON, J., AND GREEP, R. O.: The effect of alterations in thyroid function on the pituitary growth hormone content and acidophil cytology. Endocrinology, *61:* 158 (1959).
121. STAUN, H.: Various factors affecting number and size of muscle fibers in the pig. Acta Agr. Scand., *13:* 293 (1963).
122. STEARNS, G., NEWMAN, K. J., MCKINLEY, J. B., AND JEANS, P. C.: The protein requirements of children from one to 10 years of age. Ann. N.Y. Acad. Sci., *69:* 857 (1958).
123. STOCKDALE, F. E., AND HOLTZER, H.: DNA synthesis and myogenesis. Exp. Cell. Res., *24:* 508 (1961).
124. TALBERT, J. L., AND HALLER, J. A.: Muscle biopsy technique in infants and children. In: D. B. Cheek: Human Growth, p. 649 (Lea and Febiger, Philadelphia, 1968).
125. TELLO, J. F.: Die Entstehung der motorischen und sensiblen nervendigungen. I. In dem lokomotorischen System der hoheren Wirbeltiere. Muskulare Histogenese. Z. Anat. Entwicklungsgesch., *61:* 381 (1922).
126. VENDRELY, R.: The deoxyribonucleic acid content of the nucleus. In: E. Chargaff and J. N. Davidson: The Nucleic Acids, Vol. 2, p. 155 (Academic Press, New York, 1955).
127. VERNADAKIS, A., AND WOODBURY, D. M.: Electrolyte and nitrogen changes in skeletal muscle of developing rats. Amer. J. Physiol., *206:* 1365 (1964).
128. WALLS, E. W.: The microanatomy of muscle. In: G. H. Bourne: The Structure and Function of Muscle, p. 21 (Academic Press, New York, 1960).
129. WALSER, M., AND BARRAT, T. M.: Extracellular fluid in individual tissues and in whole animals: The distribution of radio-sulfate and radiobromide. J. Clin. Invest., *48:* 56 (1969).
130. WALSER, M., SELDIN, D. W., AND GROLLMAN, A.: Radiosulfate space of muscle. Amer. J. Physiol., *176:* 322 (1954).
131. WATERLOW, J. C.: Creatinine excretion. In: H. N. Munro and J. R. Allison: Mammalian Protein Metabolism, Vol. 3 (Academic Press, New York, 1969).
132. WELCKER, H., AND BRANDT, A.: Gewichtswerte de korperorgane bie dem menschen und den tieren. Arch. Anthropol., *28:* 1 (1903).
133. WIDDOWSON, E. M., AND MCCANCE, R. A.: Some effects on accelerating growth. I. General somatic development. Proc. Roy. Soc., *152:* 188 (1960).
134. WINICK, M., AND NOBLE, A.: Cellular response in rats during malnutrition at various ages. J. Nutr., *89:* 300 (1966).
135. WINICK, M., AND NOBLE, A.: Cellular response with increased feeding in neonatal rats. J. Nutr., *91:* 179 (1967).
136. WIRSEN, C., AND LARSSON, K. S.: Histochemical differentiation of skeletal muscle in foetal newborn mice. J. Embryol. Exp. Morphol., *12:* 759 (1964).
137. WOHLFART, G.: Uber das Vorkommen verschiedener Arten von Muskelfasern in der Skelettmuskulatur des Menschen und einiger Saugetiere. Acta Psychiat. Neurol. Scand. Suppl., *12:* 1 (1937).
138. YOUNG, V. R., AND ALEXIS, S. D.: In vitro activity of ribosomes and RNA content of skeletal muscle in young rats fed adequate or low protein. J. Nutr., *96:* 255 (1968).
139. YOKOYAMA, H. O., JENNINGS, R. B., AND WARTMAN, W. B.: Intercalated disks of dog myocardium. Exp. Cell. Res., *23:* 29 (1961).
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