Early life undernutrition in rats

1. Quantitative histology of skeletal muscles from underfed young and refed adult animals

BY K. S. BEDI*

Department of Child Health, Manchester University, Manchester M13 9PT

AND A. R. BIRZGALIS AND M. MAHON

Department of Anatomy, Manchester University, Manchester M13 9PT

AND J. L. SMART

Department of Child Health, Manchester University, Manchester M13 9PT

AND A. C. WAREHAM

Department of Physiology, Manchester University, Manchester M13 9PT

(*Received 7 September 1981 – Accepted 26 November 1981*)

1. Male rats were undernourished either during the gestational and suckling periods or for a period of time immediately following weaning. Some rats were killed at the end of the period of undernutrition; others were nutritionally rehabilitated for lengthy periods of time before examination. Two muscles, the extensor digitorum longus (EDL) and soleus (SOL) were studied from each rat. Histochemically-stained transverse sections of these muscles were used to determine total number of fibres, the fibre cross-sectional areas and the relative frequency of the various fibre types.

2. All rats killed immediately following undernutrition showed significant deficits in body-weight, muscle weight and fibre cross-sectional area compared to age-matched controls.

3. Animals undernourished during gestation and suckling and then fed normally for 5 months showed persistent and significant deficits in body-weight, muscle weight and total fibre number. There were also significant deficits in mean fibre cross-sectional area of each fibre type except for red fibres in the EDL. No difference in the volume proportion of connective tissue was found.

4. Rats undernourished after weaning and then fed *ad lib.* for approximately 7 months had normal body- and muscle weights. Their muscles showed no significant differences in total fibre number, relative frequency of the various fibre types, fibre size or volume proportion of connective tissue.

5. These results indicate that, although the effects on rat skeletal muscle of a period of undernutrition after weaning can be rectified, undernutrition before weaning causes lasting deficits.

There is some evidence that adult animals which have suffered a period of undernutrition early in life show impairment of performance in tests of motor co-ordination (reviewed by Smart, 1979; Smart & Bedi, 1982). This has usually been attributed to the stunting of cerebellar growth by early undernutrition. Certainly nutritional deprivation during the period of the brain growth spurt restricts cerebellar growth more than that of the rest of the brain, such that previously-undernourished adult animals show greater deficits in cerebellar weight and cellularity than are found in the other major parts of the brain (Culley & Lineberger, 1968; Chase *et al.* 1969; Dobbing & Sands, 1971). More recently there has been investigation of the effects of early undernutrition on muscle development, and the evidence from preliminary studies is not just of deficits in muscle weight, but of lasting alterations in the relative proportions of muscle fibre types (see p. 418). It has been suggested that one of the functional consequences of these muscular differences may be a greater

* Present address: Department of Anatomy, Aberdeen University, Aberdeen AB9 1AS.

susceptibility to fatigue, which may in turn be a contributory factor in the reported impairments in motor performance (Jordan *et al.* 1979).

The studies reported in the ensuing three papers were an attempt to investigate this question in an interdisciplinary fashion. The following characteristics of previously-undernourished rats were examined: (1) quantitative muscle histology, (2) whole muscle physiology, (3) motor behaviour in a revolving drum test.

Undernutrition of animals during early life, depending on its timing, may cause a failure of normal bodily growth which is resistant to nutritional rehabilitation (Widdowson *et al.* 1960; Winick & Noble, 1966; Dobbing, 1976). For a given species the timing, duration and severity of the undernutrition is critical in determining whether its effect on body-weight is permanent. This 'vulnerable period' (Dobbing, 1974) for rats occurs during the suckling period; undernutrition before weaning causes permanent stunting, whilst after weaning this effect can generally be reversed.

Skeletal muscle is a major component of body-weight. Relatively few quantitative histological studies have been carried out on skeletal muscle from rats previously undernourished during these early stages of life, although there is a considerable literature on the muscles of animals undernourished during adult life (see Table 1).

Histochemical staining methods can be used to classify skeletal muscle fibres into several different types (see p. 422 and Table 2). Several reports have investigated whether undernutrition has a differential effect on the various fibre types. For instance, Haltia *et al.* (1978) reported that the extensor digitorum longus (EDL) muscle of 180-d-old rats previously-undernourished from conception to 30 d of age had a deficit in the relative number of type I fibres with a corresponding increase in type IIB fibres compared with age-matched controls. In addition they report a deficit in the cross-sectional area of all fibre types in the previously-undernourished rats.

Howells *et al.* (1978) studied the anterior tibialis and soleus muscles from 36-week-old rats previously-undernourished from early gestation to 25 d of age. Types I and IIB fibres in the anterior tibialis of the previously-undernourished rats had deficits in mean cross-sectional area compared to controls. They also observed a deficit in the proportions of type I and IIA fibres with a corresponding increase in type IIB. In contrast, soleus muscle from previously-undernourished rats showed a significant increase in the cross-sectional area of its type IIA fibres with no changes in the proportion of its two fibre types (i.e. types I and IIA).

In preliminary experiments we too found a significant deficit in the cross-sectional area of 'white' (type IIB) fibres from the EDL muscle of adult rats previously undernourished during gestation and the suckling period (Bedi *et al.* 1978). We have now repeated and extended the scope of these experiments. We have been particularly interested in determining any differential effects of the timing of different periods of undernutrition on muscle development. Secondly, we wished to determine whether any effects were permanent. Therefore we have carried out a quantitative histological and histochemical study of muscles from rats undernourished either before or just after weaning. Some rats were killed immediately following the period of undernutrition, others were nutritionally rehabilitated for lengthy periods of time before examination.

MATERIALS AND METHODS

Animals

The rats used in this study were of the black-and-white hooded Lister strain. Virgin females were housed with males; the presence of sperm in vaginal smears indicated day 0 of pregnancy. After mating the females were housed singly. They and their offspring were treated as outlined later (p. 420).

Table 1. *Reports in the literature on the effects of experimental nutritional deprivation on muscle histology*

Authors	Timing of undernutrition and age when animals killed	Muscles studied	Results
Rowe (1968)	Adult mice killed immediately after period of 21 days undernutrition	ANTIB, biceps brachii, EDL, SOL and sterno-mastoideus	Total fibre numbers not affected. Mean fibre diameters smaller in all muscles (except soleus) due to decrease in the proportions of large diameter fibres
Goldspink & Ward (1979)	Adult mice and hamsters under-nourished for periods up to 59 d. One group of hamsters nutritionally rehabilitated for 30 d	Biceps brachii and SOL	ATPase-high fibres show decrease in size after undernutrition. These deficits disappeared after nutritional rehabilitation
Stickland, Widdowson & Goldspink (1975)	1- and 2-year-old pigs under-nourished from 10 d of age	Flexor digiti V brevis	Total fibre numbers not affected. Significant deficits in mean fibre size
Hansen-Smith, van Horn & Maksud (1978)	10-week-old rats killed immediately after 5 weeks of undernutrition	Quadriceps	Fibres from 'white' portion of muscle were smaller in undernourished animals; fibres from 'red' portion were not
Haltia, Berlin, Schucht & Sourander (1978)	Rats undernourished from conception to various ages up to 180-d-old. One group nutritionally rehabili-tated between 30 and 180 d of age	EDL	Type I and IIB fibres show permanent deficit in size and alterations in relative proportions
Howells, Mathews & Jordan (1978)	Rats undernourished from early gestation to weaning and then nutritionally rehabilitated until 180 d of age	ANTIB	Type I (intermediate) and IIB (white) fibres show permanent deficit in size. Proportion of type IIB fibres increased with concomitant deficits in type I and IIA fibres
		SOL	No permanent alterations in fibre size or proportions
Howells, Hulme & Jordan (1979)	Male and female rats undernourished from early gestation to weaning and then nutritionally rehabili-tated until 20 weeks of age	EDL, SOL	Male and female muscles show differential effects due to undernutrition. Only male EDL muscles show deficits in weight and fibre cross-sectional area. Fibres in male SOL show significant hypertrophy
Hegarty & Kim (1980)	Weanling rats undernourished until they showed 40% drop in body-weight; some rehabilitated until they were back to original body-weight	Plantaris, SOL and biceps brachii	No permanent deficits in number or size of fibres

EDL, Extensor digitorum longus; SOL, Soleus; ANTIB, Anterior tibialis.

Gestational and lactational undernutrition

The pups in this group were undernourished during the gestation and lactation periods (G^-L^- group). This was accomplished by underfeeding their mothers throughout pregnancy and lactation. They received an amount of normal food (Porton Mouse Diet) corresponding to approximately half that eaten by mothers fed *ad lib.* This restricted ration was 10 g/d during pregnancy, 15 g from day 1 to day 7 of lactation, 20 g from day 8 to day 15 and 25 g from day 16 to day 27. All animals had free access to water throughout the experiment. At birth all litters were standardized to eight pups, containing where possible six males and two females.

On postnatal day 27, seven control and seven undernourished males, none of whom were siblings, were killed for histological examination. Other males were housed singly and given food *ad lib.* until they were 180 d of age, when they and their age-matched controls were killed.

Post-weaning undernutrition

These animals (W^- group) were well-nourished before weaning (25 d) and for 5 further d. For a period of 30 d thereafter they were underfed (i.e. from postnatal day 30 to day 60). They were given 3·5 g of food daily from day 30 to day 35, 4 g from day 35 to day 40, 5 g from day 40 to day 45 and 6 g from day 45 to day 60. Groups of control and experimental male rats were killed on day 60 for histological examination. Others were nutritionally rehabilitated with an *ad lib.* supply of food until day 280 when they were also killed. All animals had free access to water throughout the experiment.

Muscles

Each rat was killed in chloroform vapour and weighed. The EDL and soleus (SOL) muscles from each hind-limb were removed in a standardized fashion and weighed; those from the right limb were placed in Bouin's fixative and later processed for embedding in plastic (2-hydroxymethacrylate). Sections from these blocks were stained with a modified trichrome stain and later used for counting total fibre number in the mid-belly regions of the muscles.

The mid-belly regions of the EDL and SOL muscles from the left limb were cut out, orientated for transverse sectioning and rapidly frozen in dichlorodifluoromethane (Arcton) cooled to its melting point of $-158°$ with liquid nitrogen. Each microtome chuck was mounted with a pair of muscles, one each from an experimental animal and its corresponding control. This ensured that each pair of control and experimental muscles was treated in exactly the same fashion. Transverse sections (10 μm thick) were cut on a Slee cryostat at a temperature of $-20°$. Serial sections from each muscle were stained histochemically with one of three methods for: succinate dehydrogenase (*EC* 1.3.99.1, SDH) (Nachlas *et al.* 1955), phosphorylase *EC* 2.4.1.1 (Takeuchi & Kuriaki, 1955), both acid- and alkali-stable myofibrillar adenosine triphosphatase (*EC* 3.6.1.3; ATPase) (Padykula & Herman, 1955; Guth & Samaha, 1970).

Morphometry

Fibre identification. From sections stained for SDH, muscle fibres were classified into three main groups (red, intermediate or white) using the criteria defined by Stein & Padykula (1962). For several muscles, fibres were further subdivided into oxidative or glycolytic and histochemically 'fast' or 'slow' (see Table 2). This allowed us to compare our fibres with those of other workers quoted in this report who base their classification on the ATPase stain and adopt the nomenclature, types I, IIA and IIB fibres (see Brooke & Kaiser, 1970 and Table 2). It should be noted however that the equivalence of these classifications may vary from muscle to muscle and between species.

Table 2. *Histochemical profiles of rat muscle fibres*

Stain...	EDL				SOL	
SDH pattern*...	Red		INT	White	INT	Red
SDH-phosphorylase...	Oxid	Oxid-glyc	Oxid-glyc	Glycolytic	Oxidative	Oxid-glyc
ATPase†...	'slow'	'fast'	'fast'	'fast'	'slow'	'fast'
Equivalent classification‡	I	IIA	IIA	IIB	I	IIA
Percentage of total	1·5	47·5	19	32	93	7

EDL, extensor digitorum longus; SOL, soleus; INT, intermediate; SDH, succinate dehydrogenase; oxid, oxidative; oxid-glyc, oxidative-glycolytic.
* Stein & Padykula (1962).
† Guth & Samaha (1969).
‡ Close (1972), Peter *et al.* (1972).

In some of the experiments all the fibres within a given muscle section were classified. In later experiments on the EDL a strategy which involved sampling from four regions located on a ventrolateral-dorsomedial axis was devised. This was found to give very similar results to those obtained when whole sections were used.

Fibre cross-sectional areas. Sections stained by the SDH method were used. For EDL muscle sections four regions located on a ventrolateral-dorsomedial axis (of the hind-limb) were photographed on a Vickers M41 light microscope. A stage graticule was used as a magnification standard. Photomicrographs were produced to a final magnification of $\times 400$. The cross-sectional areas of forty randomly-selected fibres were measured in each region. This was done with the aid of a compensating polar planimeter (Albrit) which had been determined to have a precision of better than $\pm$ 1%.

As the fibre distribution in the SOL was more homogeneous only thirty randomly-selected fibres were measured from each muscle. Each fibre measured was also identified as to type (i.e. red, intermediate or white).

Total fibre number counts. Methacrylate-embedded sections of the muscles were used for this study. Pilot experiments had revealed that methacrylate-embedded tissue was ideally suited for counts of total fibre number. This was for two reasons. First, transverse sections of the whole of each muscle could easily be obtained from these blocks. This is not always possible with frozen sections where groups of muscle fibres sometimes drop away from the section edge. Secondly, the dehydration stage of the embedding procedure caused the muscle fibres to shrink and therefore become slightly separated from each other (see Fig. 2). This facilitated the counting.

Counts were performed on transverse sections taken from the mid-belly portion of each muscle. Pilot experiments had revealed that there was little variation in the counts of total fibre number between several sections taken from such a region of a given muscle. For example, a series of six transverse sections cut at intervals of approximately 100 μm from the mid-belly region of an EDL muscle had a mean ($\pm$SE) fibre number count of 2839 ± 33. This differed significantly from sections taken from near the ends of the same muscle which had fibre counts of 2170 ± 164 and 2529 ± 97.

Volume proportion of connective tissue. The proportion of the SDH-stained muscle sections which was connective tissue (its volume fraction, V_v) was estimated by 'point count' analysis (Weibel, 1969).

Statistics. Initially, the mean values for the various measurements investigated were calculated for each rat. These were then used to obtain means ($\pm$SE) for the various groups of rats. Differences between groups were examined using Student's t test.

RESULTS

Fibre types

In the EDL the relative frequency of red, intermediate and white fibres was 49, 19 and 32% respectively. Pilot experiments showed a gradual increase in the proportion of red fibres from lateral to medial aspects of the muscle (Plate 1). ATPase staining revealed the majority of fibres (98·5%) to be histochemically 'fast' and both our intermediates and most reds to be equivalent to other workers' type IIA fibres (Table 2).

In the SOL 93% of the fibres were 'slow' intermediates (I) and 7% were 'fast' reds (IIA). No white (IIB) fibres were present (Table 2).

Our findings on the relative frequencies of fibre types present in EDL and SOL are in general agreement with those of other workers (see Pullen, 1977*b*; Howells *et al.* 1978; Soukup *et al.* 1979) and correspond with the designation of these muscles as 'fast' and 'slow' twitch respectively (see also Wareham *et al.* 1982).

Gestational and lactational undernutrition

Body, brain and muscle weights. At 27 d of age the undernourished rats in this group had a 67% deficit in body-weight compared to controls (Table 3). The EDL muscle showed an even greater deficit in weight (approximately 77%). This resulted in a significant deficit of approximately 34% in EDL muscle:body-weight (Table 3). Following nutritional rehabilitation for a period of 155 d the previously-undernourished rats had persisting deficits of approximately 22–26% in body-weight and EDL and SOL muscle weights (Table 3). Although EDL muscle:body-weight remained significantly different from that of age-matched controls (Table 3) SOL:body-weight did not do so.

Both the forebrain (brain minus cerebellum) and the cerebellum showed significant persisting deficits in weight (Table 3). The cerebellum showed a greater deficit (26%) than did the rest of the brain (12%).

Fibre cross-sectional areas. Histological examination of SDH-stained transverse sections of the mid-belly regions of these muscles revealed that undernutrition caused deficits in fibre cross-sectional areas (Plates 2 and 3). The EDL muscle fibres from 27-d-old rats killed immediately after the period of undernutrition had a 65–71% deficit in this measurement (Table 4). Following nutritional rehabilitation only white and intermediate fibres showed significant permanent deficits in fibre size compared to controls (Table 4, Plate 4).

The SOL muscle, which as previously mentioned, is almost entirely composed of intermediate fibres (Plate 5) also showed a persisting deficit in fibre cross-sectional area in these rats (Table 4). This deficit of 42% ($P < 0.01$) was greater than the 23% deficit found in the intermediate fibre cross-sectional area from the EDL muscle.

Fibre number and relative frequency estimates. The 27-d-old rats, particularly those which had been undernourished, had muscles with immature fibres (Plate 3). As the boundaries of these fibres were not always clearly visible, no reliable estimates of total fibre number could be made.

Table 3. *Body, brain and muscle weights at 27 and 180 d of age of well-fed controls (C) and of rats undernourished during gestation and lactation (G^-L^-)*

(Mean values with their standard errors)

		C			G^-L^-		
	Age (d)	*n*	Mean	SE	*n*	Mean	SE	Percentage difference
Body-wt (g)	27	6	66·0	2·5	6	21·6	3·6	−67**
	180	14	451·4	9·6	14	331·8	9·1	−26**
Forebrain wt† (mg)	27	—	—	—	—	—	—	—
	180	14	1354	20	14	1197	20	−12**
Cerebellum wt (mg)	27	—	—	—	—	—	—	—
	180	14	331	12	14	244	13	−26**
EDL wt (mg)	27	6	30·0	2·3	6	6·9	1·9	−77**
	180	14	190·8	2·8	14	148·6	4·7	−22**
EDL:body-wt ($\times 10^{-3}$)	27	6	0·452	0·024	6	0·300	0·028	−34**
	180	14	0·424	0·008	14	0·450	0·008	+6*
SOL wt (mg)	27	—	—	—	—	—	—	—
	180	14	162·4	4·0	14	123·7	3·1	−24**
SOL:body-wt ($\times 10^{-3}$)	27	—	—	—	—	—	—	—
	180	14	0·361	0·006	14	0·374	0·008	+4

EDL, extensor digitorum longus; SOL, soleus.

* $P < 0.05$, ** $P < 0.01$.

† Brain minus cerebellum.

Table 4. *EDL and SOL cross-sectional areas (μm^2) at 27 and 180 d of age of well-fed controls (C) and of rats undernourished during gestation and lactation (G^-L^-)*

(Mean values with their standard errors)

	Age (d)	C			G^-L^-			Percentage difference
		n	Mean	SE	*n*	Mean	SE	
EDL:								
Red	27	6	355	19	6	126	20	−65**
	180	8	1925	104	8	1683	104	−13
White	27	6	678	49	6	197	27	−71**
	180	8	5314	293	8	4049	216	−24**
Intermediate	27	6	534	30	6	182	21	−66**
	180	8	3375	183	8	2592	273	−23*
Mean of all fibres	27	6	522	40	6	167	15	−68**
	180	8	3538	182	8	2770	109	−22**
SOL:								
Mean of all fibres	27	—	—	—	—	—	—	—
	180	8	4183	233	8	2409	165	−42**

EDL, extensor digitorum longus; SOL, soleus.
* $P < 0.05$, ** $P < 0.01$.

The EDL muscles (Plate 4) from the 180-d-old control rats had a greater total fibre count ($P < 0.01$) than the EDL muscles from the previously undernourished group of animals (Table 5). In addition to this deficit in total fibre number the relative frequency of the fibre types differed. Thus previously-undernourished rats tended to have a greater frequency of red fibres, with a concomitant deficit in white fibres (Fig. 1) compared to controls. The relative frequency of intermediate fibres was not altered significantly.

The SOL muscle (Plate 5) also showed a persisting deficit of approximately 19% ($P < 0.01$) in total fibre number (Table 5). However, no permanent changes in the relative frequency of its two fibre types were found.

Table 5. *Total muscle fibre number and volume proportion (V_v) of connective tissue at 180 d of age of well-fed controls (C) and of rats undernourished during gestation and lactation (G^-L^-)*

(Mean values with their standard errors)

	C			G^-L^-			Percentage difference
	n	Mean	SE	*n*	Mean	SE	
EDL:							
Total fibre number	8	2848	48	8	2260	41	−21**
V_v connective tissue (%)	8	6·4	0·4	8	6·7	0·5	+5
SOL:							
Total fibre number	8	2727	58	8	2218	73	−19**
V_v connective tissue (%)	8	9·7	0·6	8	10·6	0·6	+9

EDL, extensor digitorum longus; SOL, soleus.
** $P < 0.01$.

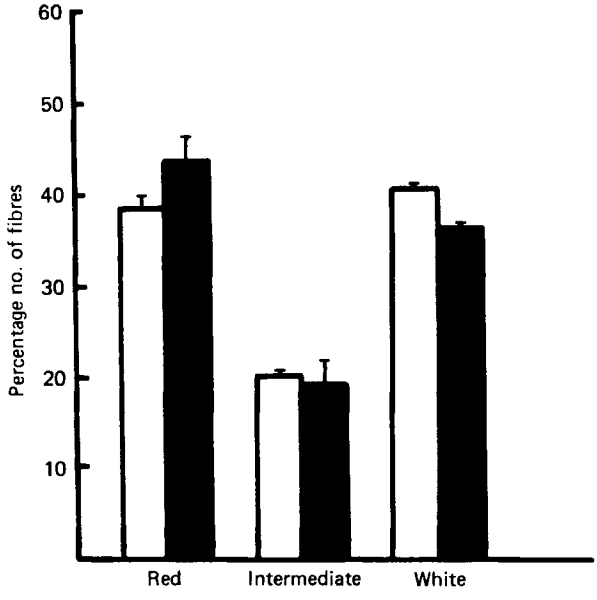


Fig. 1. Relative frequency of the three fibre types in whole extensor digitorus longus (EDL) muscle cross-sections from three control (□) and three refed, previously-undernourished (■), $G^{-}L^{-}$ animals at 180 d of age. No statistical differences were found for red or intermediate fibres whereas significant alterations ($P < 0{\cdot}01$) were found in the white group. (Similar results were found for a group of eight control versus eight experimental animals where only 200 fibres were sampled from each muscle). Values are means with their standard errors represented by vertical bars.

Table 6. *Body-, and muscle weights at 60 and 280 d of age of well-fed controls (C) and of rats undernourished after weaning (W^{-})*

(Mean values with their standard errors)

		C			W^{-}		
	Age (d)	*n*	Mean	SE	*n*	Mean	SE	Percentage difference
Body-wt (g)	60	7	272·2	5·6	5	81·5	2·9	−70**
	280	7	540·3	18·1	7	509·4	15·6	−6
EDL wt (mg)	60	7	123·6	4·1	5	36·7	2·6	−68**
	280	7	196·7	7·3	7	177·3	11·1	−10
EDL:body-wt ($\times 10^{-3}$)	60	7	0·46	0·01	5	0·48	0·02	+4
	280	7	0·37	0·01	7	0·35	0·01	−5
SOL weight (mg)	60	—	—	—	—	—	—	—
	280	7	157·4	5·3	7	161·9	8·9	−8
SOL:body-wt ($\times 10^{-3}$)	60	—	—	—	—	—	—	—
	280	7	0·33	0·01	7	0·32	0·01	−3

EDL, extensor digitorum longus; SOL, soleus.
** $P < 0{\cdot}01$.

Table 7. *EDL and SOL fibre cross-sectional areas (μm^2) at 60 and 280 d of age of well-fed controls (C) and of rats undernourished after weaning (W^-)*

(Mean values with their standard errors)

		C			W^-			
	Age (d)	*n*	Mean	SE	*n*	Mean	SE	Percentage difference
EDL:								
Red fibres	60	7	1336	57	7	699	44	−48**
	280	7	1653	186	7	1579	187	−5
White fibres	60	7	3254	17	7	1304	89	−60**
	280	7	5067	654	7	4534	417	−11
Intermediate fibres	60	7	2116	92	7	1008	75	−52**
	280	7	2930	310	7	2733	316	−7
Mean of all fibres	60	7	2233	100	7	1004	59	−55**
	280	7	3217	379	7	2949	271	−8
SOL:								
Mean of all fibres	60	—	—	—	—	—	—	—
	280	7	2862	113	7	2807	129	−2

EDL, extensor digitorum longus; SOL, soleus.
** $P < 0.01$.

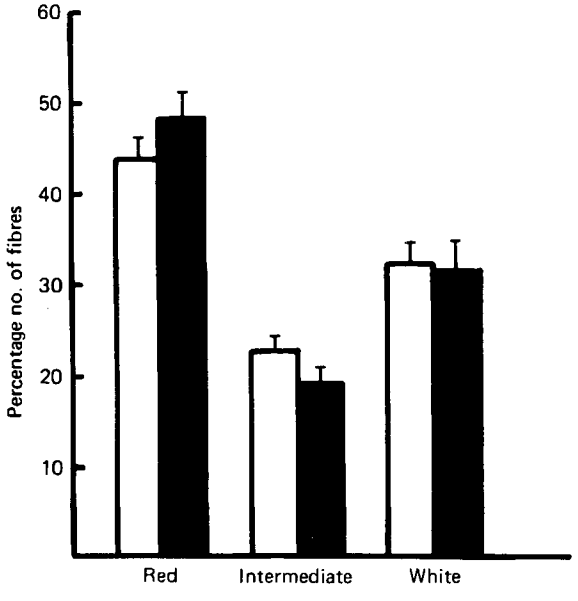


Fig. 2. Relative frequency of the three fibre types in extensor digitorus longus (EDL) muscle from seven control (□) and five refed, previously-undernourished (■), W^- animals. None of the differences in percentage number of red, intermediate or white fibres was statistically significant. Values are means with their standard errors represented by vertical bars.

Table 8. *Total muscle fibre number and volume proportion* (V_v) *of connective tissue at 280 d of age of well-fed controls* (*C*) *and of rats undernourished after weaning* (W^-)

(Mean values with their standard errors)

	C			W^-			Percentage difference
	n	Mean	SE	*n*	Mean	SE	
EDL:							
Total number of fibres	7	2599	33	7	2677	45	+3
V_v connective tissue	7	7·8	1·7	7	7·2	1·3	−8
SOL:							
Total number of fibres	7	2014	94	7	1951	91	−3
V_v connective tissue	7	10·2	0·9	7	10·9	1·6	+7

EDL, extensor digitorum longus; SOL, soleus.

There were no significant differences between control and previously-undernourished rats in the proportion of the muscle sections occupied by connective tissue (Table 5).

Post-weaning undernutrition

Body- and muscle weights. At 60 d of age the undernourished rats in this group had deficits of 70 and 68% in body- and EDL muscle weights respectively compared with age-matched controls (Table 6); however, there was no significant difference in EDL muscle: body-weight in these animals compared to the controls. Following nutritional rehabilitation, until 280 d of age, there were no statistically-significant deficits in any of these measurements, nor in the measurements relating to SOL (Table 6).

Fibre cross-sectional areas. The EDL muscle fibres from 60-d-old rats killed immediately after the period of undernutrition had large deficits in cross-sectional area (Table 7). These deficits had largely disappeared after nutritional rehabilitation.

The SOL muscle also showed no persisting deficit in fibre cross-sectional area in these animals.

Fibre number and relative frequency estimates. There was no statistically-significant, permanent alteration in either the total number or in the relative amounts of the various fibre types in the muscles obtained from the previously-undernourished rats of this group (Fig. 2; Table 8). Nor were there any significant differences in the volume proportion of connective tissue between control and previously-undernourished rats for either EDL or SOL (Table 8).

DISCUSSION

In our experiments rats which had been undernourished during the gestation and suckling periods had muscles which showed deficits in weight, fibre number and fibre size. Nutritional rehabilitation over a lengthy period did not restore these deficits to normal. On the other hand, rats which were undernourished for a period just after weaning and then refed for approximately 8 months, showed no such permanent deficits. This strongly suggests that, like the brain, muscle tissue has a period during which its development is particularly vulnerable to undernutrition. Undernutrition during this period caused lasting deficits and distortions of muscle structure. Such effects may well be permanent.

It is possible that human muscle development is similarly vulnerable. Montgomery (1962) has described gross alterations in muscle histology, including decreased fibre number, in undernourished infants. More recently Hansen-Smith *et al.* (1979) have found that previously-malnourished Jamaican infants had persisting deficits in muscle size even after 'clinical recovery' as defined by the attainment of expected weight-for-height.

There are several reports in the literature on the effects of nutritional deprivation on muscular tissue. Of these, some have concentrated on gross changes or biochemical estimates or both of such factors as, total DNA content of individual muscles (e.g. Winick & Noble, 1966; Widdowson, 1970; Dickerson *et al.* 1972; Williams & Hughes, 1978). There have been relatively few previous studies on the histological structure of muscles obtained from undernourished animals (see Table 1). It is not always possible to compare directly the results presented in these reports with our own. This is due to differences in the timing of the imposed period of undernutrition, the species and muscle studied, and to the different analytical methods adopted. For instance, some of the studies have examined muscles only immediately after the period of undernutrition, without follow-up investigations to assess the effectiveness of nutritional rehabilitation (e.g. Rowe, 1968; Stickland *et al.* 1975; Hansen-Smith *et al.* 1978). Others have reported only on animals which had been undernourished after weaning (Goldspink, 1973; Rowe, 1968; Hansen-Smith *et al.* 1978; Goldspink & Ward, 1979; Hegarty & Kim, 1980). In addition, many of them have not employed histochemical staining to distinguish between the various fibre types present. However, the general picture which emerges from these studies is that nutritional deprivation after weaning does initially affect muscle histology, mainly by a decrease in fibre size (Rowe, 1968; Goldspink, 1973; Hansen-Smith *et al.* 1978) but that the effects are not permanent (Goldspink & Ward, 1979; Hegarty & Kim, 1980). This agrees with our present observations. In only three of the studies listed in Table 1 (Haltia *et al.* 1978; Howells & Jordan, 1978; Howells *et al.* 1979) were muscles investigated from rats undernourished before weaning and subsequently rehabilitated. These are further discussed.

Our observations that white and intermediate fibres from the EDL muscle of rats undernourished before weaning show a permanent deficit in size whereas red fibres do not, are in agreement with our previous work (Bedi *et al.* 1978) and with other workers who have carried out similar experiments (Haltia *et al.* 1978; Howells *et al.* 1978).

In the present study, the EDL muscles of adult previously-undernourished rats had significant deficits in the relative frequency of white (type IIB) fibres, an increase in the red fibres (types I and IIA) and no significant change in the intermediate (type IIA) fibres. This is in contrast to results published by Haltia *et al.* (1978) for the EDL, and by Howells *et al.* (1978) for the similarly 'fast' tibialis anterior muscle. These workers found relative increases in the frequencies of type IIB (white) fibres and decreases in the other types in muscles of adult rats previously-undernourished before weaning. However, they do not provide statistical information on the significance of these findings. In later work, on the EDL muscle Howells *et al.* (1979) found no alteration in the relative frequencies of fibre types in their previously-undernourished rats.

These discrepancies may be due to the different muscles examined, the strain of rats used or to the differing histochemical procedures employed for fibre typing. It is also possible that the sampling of '300 adjacent muscle fibres' by Haltia *et al.* (1978) or '200 fibres' by Howells *et al.* (1978) may have been unrepresentative of the whole muscle. This is due to the heterogeneous distribution of the three fibre types in EDL and tibialis anterior (Plate 1; Pullen, 1977*a*, *b*). In the present study each and every fibre in several control and previously-undernourished EDL muscles was classified into the various types. This method is not susceptible to any vagaries of sampling technique. The results obtained are the true fibre proportions found in these muscle sections; they are not merely estimates.

Comparison of our values for the size of fibres in the SOL muscles of previously-

undernourished rats with that obtained by Howells *et al.* (1978) and Howells *et al.* (1979) also shows some discrepancies. Thus, in the present experiments we found that rats undernourished before weaning had substantial (−42%) permanent deficits in overall fibre size. It may be recalled that the majority of the fibres present in SOL are of the intermediate type. Howells *et al.* (1978) found no such permanent deficits in the SOL muscles of the rats in their experiments, and in a later study (Howells *et al.* 1979) even observed that there was some muscle fibre hypertrophy in previously-undernourished rats. There is no immediately obvious explanation for this discrepancy. Howells *et al.* (1978) put forward two possible explanations for their results. First they suggest the possibility of 'selective sparing' of fibres from physiologically-'slow' muscles during early undernutrition. Secondly they argue that there could be 'selective postnatal recovery' of fibres in the physiologically-'slow' SOL muscle but not in the 'fast' anterior tibialis muscle. Our results throw considerable doubt on both these hypotheses. Even qualitative examination of sections of SOL muscles from weanling rats undernourished from conception revealed that they were considerably affected. This argues against the idea of any 'selective sparing'. Our observation that there was a permanent deficit in the over-all fibre size in SOL is not compatible with the suggestion of 'selective postnatal recovery'.

It would appear that adequate nutrition during the period up to weaning is essential for the proper development of skeletal muscles in general. Growth of muscular tissue occurs by hyperplasia during prenatal life and almost exclusively by hypertrophy from soon after birth (see Goldspink, 1973). In addition, the early development and differentiation of fibre types is partly dependent on the nervous system which is known to be severely affected by early undernutrition (Dobbing, 1974; Thomas *et al.* 1979; Bedi, Hall *et al.* 1980; Bedi, Thomas *et al.* 1980; see also Table 3). Whether the muscle is detrimentally affected directly by these factors or by hormonal and enzymic influence during starvation (Trenkle, 1974; Frayn & Maycock, 1979; Lammi-Keefe *et al.* 1981) remains to be elucidated. Indeed, Howells *et al.* (1979) have reported sex related differences in the response of muscles to early undernutrition.

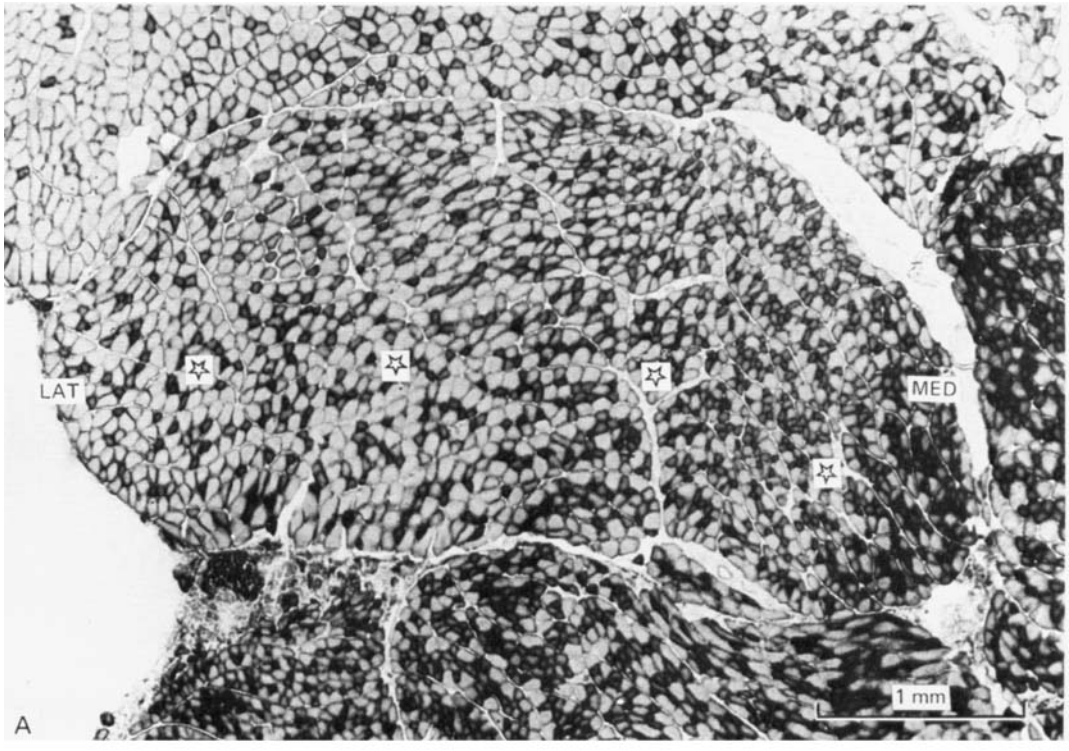
Finally, further questions may arise as to the functional implications of our present findings. Though total muscle fibre cross-sectional area was greatly reduced (EDL − 38%, SOL − 53%), without there being any change in the percentage of connective tissue, in muscles from rats refed after preweaning undernutrition, no gross differences in muscle:body-weight values were found. This may preclude any noticeable variation in locomotion or physiological measures of muscle strength unless there have been other changes (such as myofibril packing or efficiency e.g. Patterson & Goldspink 1973) not studied in the present investigation. Predictions of any modifications in over-all contraction velocity or muscle fatiguability based entirely on observations of changes in the relative proportions of the various fibre types are liable to be erroneous due to concomitant alterations in enzyme activities (Howells & Jordan, 1978; Birzgalis *et al.* 1980 and Mahon, Birzgalis and Bedi, unpublished results).

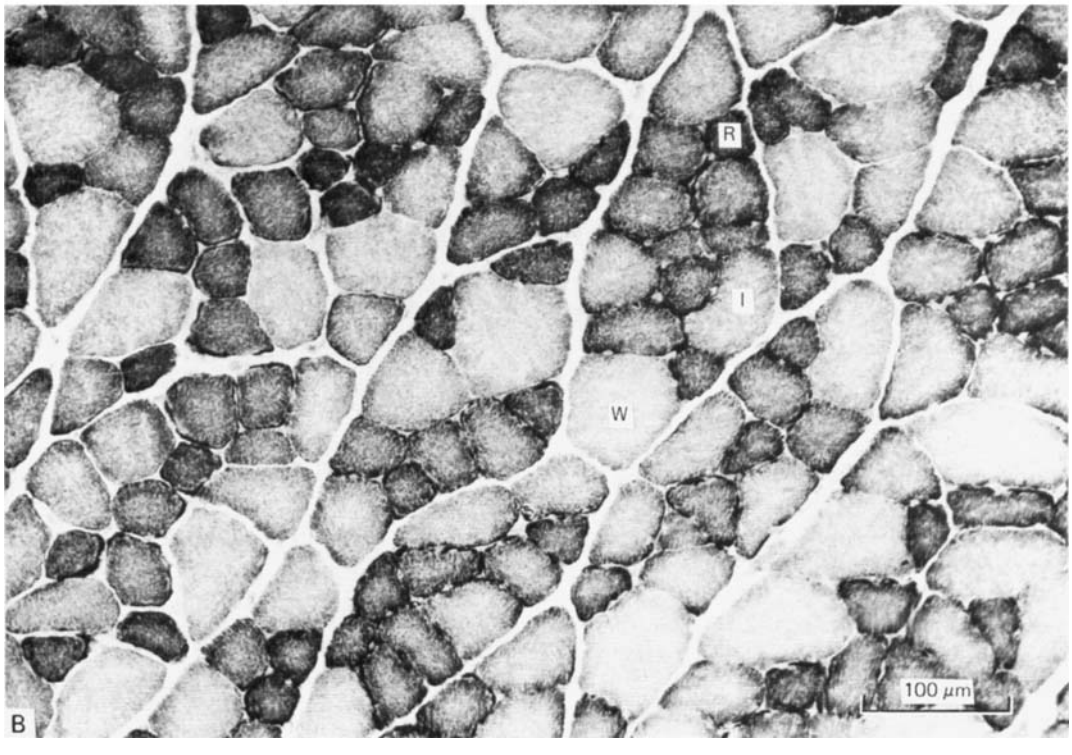
In conclusion, we have shown that undernutrition of rats during the gestation and suckling period causes lasting deficits and distortions of the histological structure of both 'fast'- and 'slow'-twitch skeletal muscles. Undernutrition outside this period shows essentially similar deficits, but these appear to be reversible. Whether these deficits and distortions are accompanied by detectable physiological and behavioural alterations were the subjects of further investigations, the results of which are reported in two further papers (Smart & Bedi, 1982; Wareham *et al.* 1982).

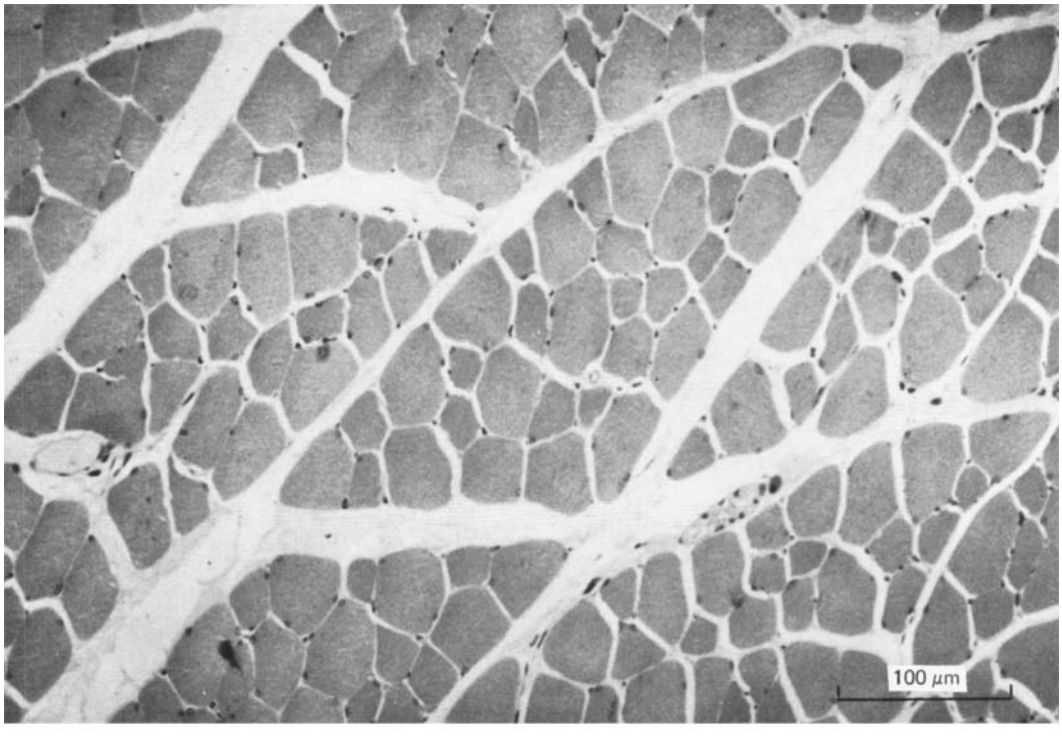
K. S. B. and J. L. S. were financed from grants to Professor John Dobbing from the Medical Research Council and the National Fund for Research into Crippling Diseases.

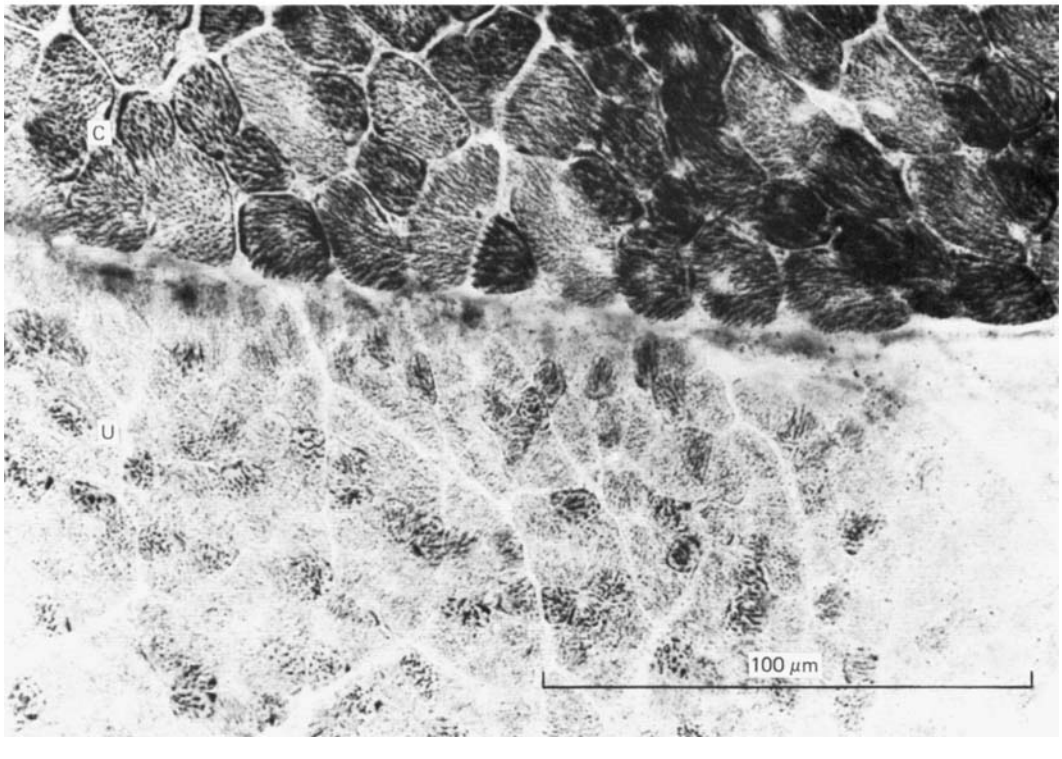
REFERENCES

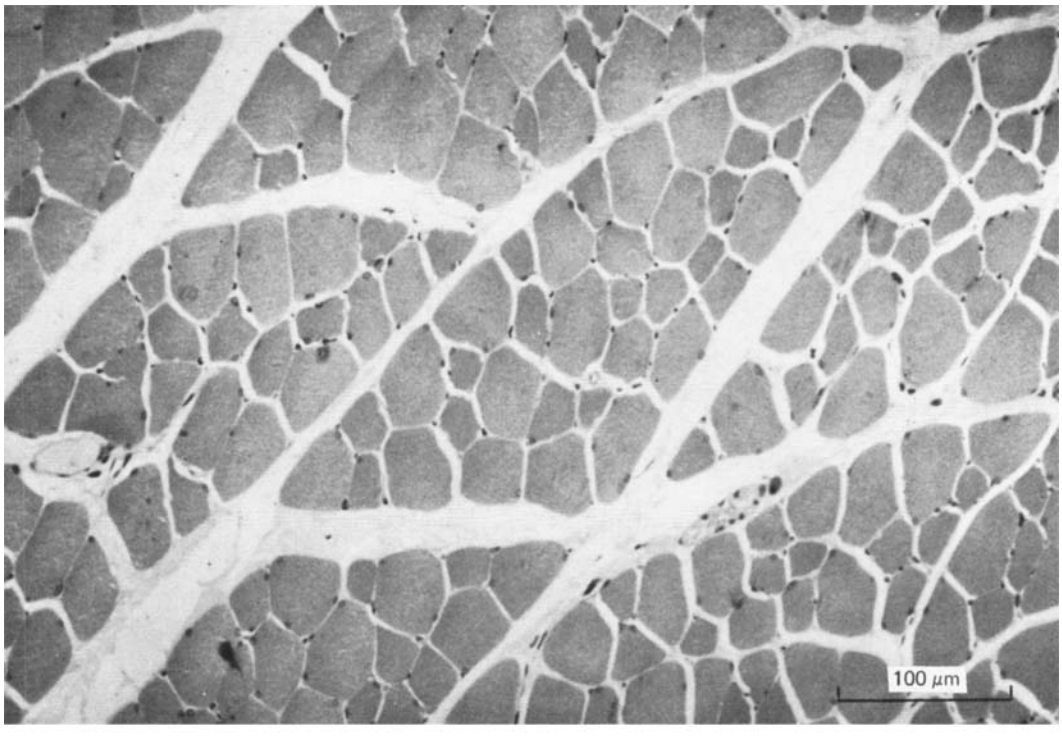
Bedi, K. S., Hall, R., Davies, C. A. & Dobbing, J. (1980). *J. Comp. Neurol.* **193**, 863.
Bedi, K. S., Mahon, M. & Smart, J. L. (1978). *J. Nutr.* **37**, 59A.
Bedi, K. S., Thomas, Y. M., Davies, C. A. & Dobbing, J. (1980). *J. Comp. Neurol.* **193**, 49.
Birzgalis, A. R., Bedi, K. S., Mahon, M. & Smart, J. L. (1980). *J. Anat.* **130**, 651.
Brooke, M. H. & Kaiser, K. K. (1970). *Arch. Neurol.* **23**, 369.
Chase, H. P., Lindsley, W. F. B. & O'Brien, D. (1969). *Nature, Lond.* **221**, 554.
Close, R. I. (1972). *Physiol. Rev.* **52**, 129.
Culley, W. J. & Lineberger, R. O. (1968). *J. Nutr.* **96**, 375.
Dickerson, J. W., Hughes, P. C. R. & McAnulty, P. A. (1972). *Br. J. Nutr.* **27**, 527.
Dobbing, J. (1974). In *Scientific Foundations of Paediatrics*, p. 565 [J. A. Davis and J. Dobbing, editors]. Philadelphia: Heinemann, London.
Dobbing, J. (1976). In *The Biology of Human Fetal Growth*, p. 137 [D. F. Roberts and A. M. Thompson, editors]. London: Taylor & Francis.
Dobbing, J. & Sands, J. (1971). *Biol. Neonat.* **19**, 363.
Frayn, K. N. & Maycock, P. F. (1979). *Biochem. J.* **184**, 323.
Goldspink, G. (1973). In *The Structure and Function of Muscle I*, p. 179 [G. H. Bourne, editor]. New York: Academic Press.
Goldspink, G. & Ward, P. S. (1979). *J. Physiol., Lond.* **296**, 453.
Guth, L. & Samaha, F. J. (1969). *Exp. Neurol.* **25**, 138.
Guth, L. & Samaha, F. J. (1970). *Exp. Neurol.* **28**, 365.
Haltia, M., Berlin, Ö., Schucht, H. & Sourander, P. (1978). *J. Neur. Sci.* **36**, 25.
Hansen-Smith, F. M., Picou, D. & Golden, M. H. (1979). *Br. J. Nutr.* **41**, 275.
Hansen-Smith, F. M., van Horn, D. L. & Maksud, M. G. (1978). *J. Nutr.* **108**, 248.
Hegarty, P. V. J. & Kim, K. O. (1980). *Br. J. Nutr.* **44**, 123.
Howells, K. F., Hulme, J. M. L. & Jordan, T. C. (1979). *Res. Exp. Med.* **176**, 137.
Howells, K. F. & Jordan, T. C. (1978). *Histochemistry* **58**, 97.
Howells, K. F., Mathews, D. R. & Jordan, T. C. (1978). *Res. Exp. Med.* **173**, 35.
Jordan, T. C., Howells, K. F. & Piggot, S. M. (1979). *Behav. Neural Biol.* **25**, 126.
Lammi-Keefe, C. J., Hegarty, P. V. J. & Swan, P. B. (1981). *Experientia* **37**, 25.
Montgomery, R. D. (1962). *J. clin. Path.* **15**, 511.
Nachlas, M. M., Tyson, K., De Souza, E., Cheng, C. & Seligman, A. M. (1955). *J. Histochem. Cytochem.* **5**, 420.
Padykula, H. A. & Herman, E. (1955). *J. Histochem. Cytochem.* **3**, 170.
Patterson, S. & Goldspink, G. (1973). *Z. Zellforsch.* **146**, 375.
Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A. & Stempel, K. E. (1972). *Biochemistry* **11**, 2627.
Pullen, A. H. (1977*a*). *J. Anat.* **123**, 1.
Pullen, A. H. (1977*b*). *J. Anat.* **123**, 467.
Rowe, R. W. D. (1968). *J. exp. Zool.* **167**, 353.
Smart, J. L. (1979). In *Chemical Influences on Behaviour*, p. 1 [K. Brown and S. J. Cooper, editors]. New York: Academic Press.
Smart, J. L. & Bedi, K. S. (1982). *Br. J. Nutr.* **47**, 439.
Soukup, T., Wydra, J. & Cerny, M. (1979). *Histochemistry* **60**, 71.
Stein, J. M. & Padykula, H. A. (1962). *Am. J. Anat.* **110**, 103.
Stickland, N. C., Widdowson, E. M. & Goldspink, G. (1975). *Br. J. Nutr.* **34**, 421.
Takeuchi, T. & Kuriaki, H. (1955). *J. Histochem. Cytochem.* **3**, 153.
Thomas, Y. M., Bedi, K. S., Davies, C. A. & Dobbing, J. (1979). *Early Hum. Develop.* **3**, 109.
Trenkle, A. (1974). *J. Anim. Sci.* **38**, 1142.
Wareham, A. C., Mahon, M., Bedi, K. S. & Smart, J. L. (1982). *Br. J. Nutr.* **47**, 433.
Weibel, E. R. (1969). *Int. Rev. Cytol.* **26**, 235.
Widdowson, E. M. (1970). In *The Physiology and Biochemistry of Muscle as a Food*, p. 511 [E. J. Briskey, R. G. Cassens and B. B. Marsh, editors]. Madison, Wisconsin: University of Wisconsin Press.
Widdowson, E. M., Dickerson, J. W. T. & McCance, R. A. (1960). *Br. J. Nutr.* **14**, 457.
Williams, J. P. G. & Hughes, P. C. R. (1978). *Acta Anat.* **101**, 249.
Winick, M. & Noble, A. (1966). *J. Nutr.* **89**, 300.

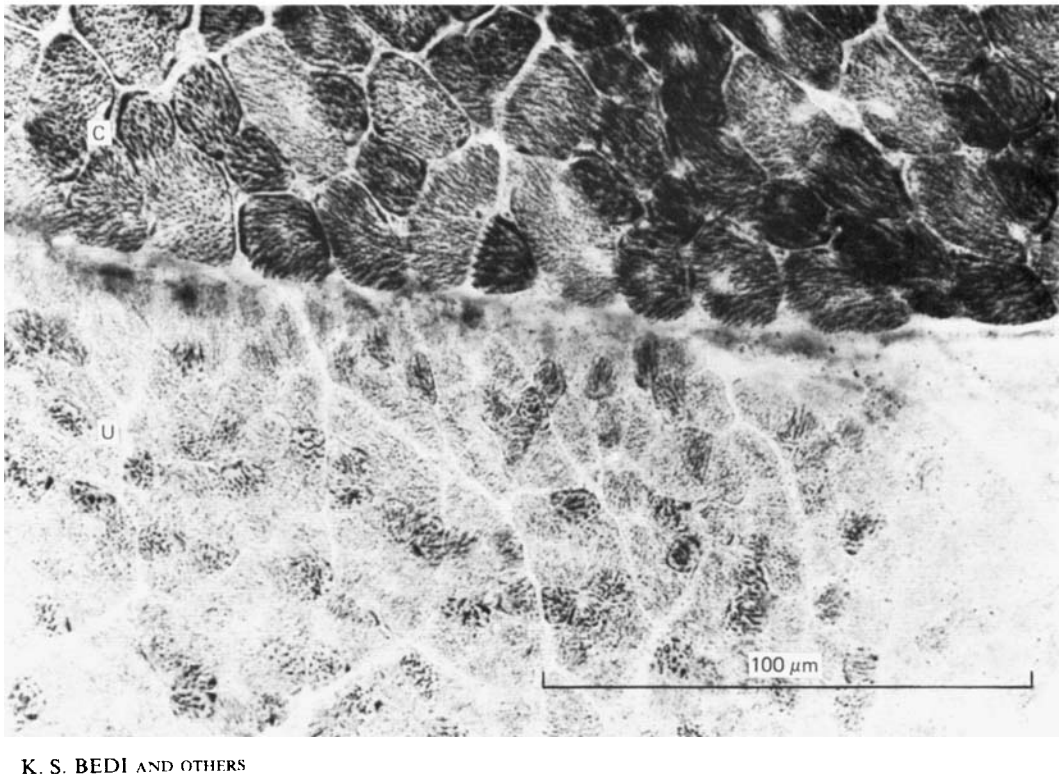












EXPLANATION OF PLATES

Plate 1. (*a*) Mid-belly cross-section of a whole extensor digitorum longus (EDL) muscle stained for succinate dehydrogenase (*EC* 1.3.99.1). Note the increase in frequency of red fibres from the lateral (LAT) to the medial (MED) aspects of the muscle and the approximate position of the four regions sampled (*). (*b*) Normal appearance of adult rat EDL muscle stained for succinate dehydrogenase showing red (R), intermediate (I) and white (W) fibres.

Plate 2. Section of methacrylate embedded EDL muscle showing the clearly-defined though slightly-shrunken fibres as used for total fibre number counts.

Plate 3. EDL muscles (succinate dehydrogenase) from control (C) and undernourished (U) G^-L^- pups at 27 d of age. The control muscle is more darkly stained and histochemically mature than the undernourished muscle.

Plate 4. EDL muscles (succinate dehydrogenase) from a control (C) and refed, previously-undernourished (U) G^-L^- rat at 180 d of age. Note the smaller fibres in the previously undernourished muscle.

Plate 5. Soleus muscle (succinate dehydrogenase) from a control (C) and refed, previously-undernourished (U) G^-L^- rat at 180 d of age showing the marked difference in fibre size.

Printed in Great Britain