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Growth and degeneration of motor end-plates in normal cat hind limb muscles

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INTRODUCTION

It has long been known that end-plates undergo growth and degeneration in a wide variety of experimental and pathological conditions (Coërs & Woolf, 1959; Zacks, 1964), but Barker & Ip (1965, 1966) were the first to demonstrate that such processes occur in the normal muscles of healthy young adult mammals. They suggested that there is a causal relationship between the growth and degeneration of end-plates such that the terminals have a limited life-span, at the end of which they degenerate and are replaced in one of three possible ways. Two of these, *rejuvenation* and *substitution*, are illustrated diagrammatically in Fig. 1. The third proposed mode was by *collateral sprouting*, a process similar to the re-innervation of muscle fibres in partially denervated muscles (Edds, 1950, 1953).

In the present study, a detailed quantitative examination was made of the growth and degeneration of motor end-plates in normal muscles of the cat hind limb. The results suggest that the two processes are not causally related as proposed in the 'replacement hypothesis' of Barker & Ip (1965, 1966), but are separate phenomena associated with ageing growth leading to an increase in complexity of the end-plates (*elaboration* – Fig. 3), and degeneration being related to loss of muscle fibres.

MATERIALS AND METHODS

The peroneus digiti quinti (PDQ) and soleus muscles of the cat hind limb were the principal objects of study. The former was selected as an example of a muscle containing all three histochemical types of muscle fibre (Barker, Stacey & Adal, 1970) and because its small size is convenient for processing in a variety of ways. The soleus muscle was selected as an example of a 'slow' muscle, in order to demonstrate differences in end-plate morphology resulting from the function of the muscle or from the histochemical types of muscle fibre.

The terminal innervation was examined in unoperated muscles from young adult cats (i.e. over 6 months old and weighing over 2·0 kg; cf. Nyström, 1968*b*), and those that were 6, 10, 15, 18 and 19 years old. The 15 and 18 year old animals were regarded as senile on the basis of their general condition and the fact that the average life-span of the cat is about 10 years (Lansing, 1959) although a 'well-supported' maximum of 31 has been reported (Comfort, 1964).

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All the animals were killed with a Nembutal (Abbot Laboratories) overdose administered by intraperitoneal (young adult and 10 year old cats) or intracardiac (6, 15, 18 and 19 year old cats) injection. The muscles were removed as soon as possible after the onset of deep surgical anaesthesia.

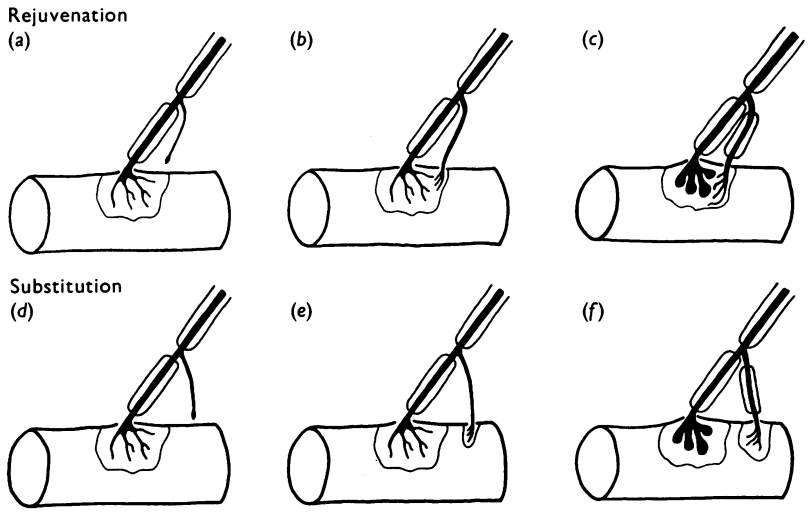

Fig. 1. The process of end-plate replacement according to Barker & Ip (1965, 1966). In *rejuvenation* (*a-c*) a nodal sprout grows towards the sole-plate of the parent ending (*a*), forms axon terminals (*b*), and finally the parent ending degenerates (*c*). In *substitution* (*d-f*) a new end-plate is substituted for the old one by a similar process involving the formation of a new sole-plate alongside the old one.

In the study of the terminal innervation in teased silver preparations, one soleus muscle was taken from a young adult cat (C254) and one from each of the 6, 10 and 18 year old cats. The PDQ muscles were from two young adult cats (C254, two muscles; C230, one muscle) and the 10 year old cat (C294, two muscles). There were no differences in the morphology of end-plates from PDQ muscles of animals of the same age, and the samples were therefore pooled.

Transverse sections of the remaining PDQ and soleus muscles of the older animals were prepared and examined for the presence of atrophic muscle fibres and other signs of abnormality. Counts of the total number of muscle fibres present were made on sections from the bellies of the PDQ muscles as well as a PDQ muscle from a third young adult cat (C283).

The terminal innervation of the gastrocnemius and soleus muscles of a kitten aged 10 weeks was examined in serial sections stained with the Holmes silver method (Carleton & Drury, 1957).

In addition to the above material, teased silver preparations of muscles removed at various intervals after section of the muscle nerve were examined. This material was prepared in connexion with other work carried out by Barker *et al.* (1970).

The intramuscular nerves and end-plates were examined in teased preparations of muscles stained by the modified De Castro silver impregnation method (Barker &

Ip, 1963) as improved by Barker *et al.* (1970). This method gives a clearer and more complete picture of the terminal innervation than the methylene blue or gold chloride techniques, and makes it possible to determine the exact points of origin and termination of fine outgrowths of the terminal axon.

In order to minimize artefacts due to variability of silver impregnation or subjective bias, only well-impregnated terminal sprays of end-plates were examined, and all the end-plates in a selected spray were included in the sample. Each sample of end-plates was examined several times during the course of the study, often at intervals of over a year. Comparisons were made between successive examinations of the same preparations, ensuring that all criteria were being rigidly adhered to.

In order to improve the clarity of the photomicrographs, several photographs were taken at different focal planes where necessary, and a montage prepared.

Measurements of end-plate and muscle fibre diameters were made using a Zeiss micrometer eyepiece. The measurements in teased silver preparations were made at a magnification of $\times 640$; those on sections stained for adenosine triphosphatase activity at $\times 400$.

The end-plate diameter was calculated as the mean of two orthogonal diameters, one of which was in the long axis of the muscle fibre. The ratio of these measurements (the axial ratio) was used as an index of the shape of the end-plate. This parameter was preferred to the ratio of the greater to the lesser diameter (L_1/L_2 ratio of Woolf, 1970) because it gave an indication of the relationship of the shape of the end-plate relative to the long axis of the muscle fibre. In practice there was little difference between the axial and L_1/L_2 ratios in this material because the end-plates were nearly always slightly elongated in the long axis of the muscle fibre.

The diameter of the muscle fibre was calculated as the mean of 3 to 5 measurements made near the end-plate. The ratio of the end-plate and muscle fibre diameters, expressed as a percentage (the percentage ratio), was also calculated. The end-plates and their muscle fibres were selected for measurement on the basis of clarity. The samples were not entirely random because specific types of end-plate were occasionally selected to increase sample sizes.

The measurements of muscle fibres and end-plates were liable to error through shrinkage and deformation of the material during preparation, but since all the measurements were made on material prepared in the same way, any comparisons made within the results were valid.

Estimates of the number of axon terminals present in each end-plate were usually made wherever possible, during both morphological examination and measuring. In the case of the PDQ muscles of the 10 year old cat, no estimates were made during the measurements and hence no direct correlation could be attempted between this parameter and the others.

In addition to the calculation of mean values and the standard error of the mean, each population of measurements was tested for skew and kurtosis using the g_1 and g_2 tests, respectively (Snedecor, 1962). The correlation coefficient (r) was calculated for each of the possible pairs of populations in a given muscle. Student's t test was used to determine the level of significance of correlation coefficients and differences between mean values.

One PDQ and one soleus muscle from a fourth young adult cat (C303) were used

to demonstrate the different histochemical types of muscle fibre. The methods used were those of Pearse (1961), Eränkö & Palkama (1961) and Guth & Samaha (1970) for the activities of succinic dehydrogenase, phosphorylase, and alkaline-stable actinomyosin adenosine triphosphatase, respectively. The muscle fibres were typed using the results of all three methods. Measurements of muscle fibre diameter were made. The results confirmed the observations of Nyström (1968*b*) on the types of muscle fibre, and of Barker *et al.* (1970) regarding the frequency of each type in PDQ. There was no correlation between muscle fibre type and diameter, the populations showing a considerable degree of overlap.

RESULTS

Terminology

As in any morphological study, it is essential that the descriptive terms used be carefully defined.

It is convenient to classify end-plates according to the number of myelinated branches of the terminal axon that contribute to their formation. The simplest type of end-plate is formed by the unbranched terminal axon, and is described as a T_1 end-plate (Fig. 2*a*). Similarly, more complex end-plates formed by two or three myelinated branches of the terminal axon are referred to as T_2 (Fig. 2*c*) and T_3 end-plates, respectively. More complex end-plates (T_4 and T_5 forms) were observed only rarely in the muscles examined.

The terminal axon usually divides at the last node to give two or more myelinated branches to the end-plate, but the division may occur more proximally. The branches may supply more or less equal numbers of axon terminals to the end-plate. The more complex end-plates (T_2–T_5 forms) are considered as single entities because of the proximity of the terminals derived from each of the branches of the terminal axon. Further, in cases where the sole-plate nuclei are stained, it can be clearly seen that there is but a single sole-plate. Examples of these different morphological type of motor end-plate are illustrated in Figs. 6–10.

If the axon terminals from each of the branches of the terminal axon lie on separate sole-plates, the ending is termed *duplex* (Fig. 2*d*). In over 4,000 end-plates examined in teased silver preparations, only five convincing examples of duplex endings were observed. No case of a muscle fibre being innervated by more than one terminal axon was encountered.

The other descriptive terms used are based on those of Barker & Ip (1966) (see their Fig. 1). Thus, a *sprout* is defined as a non-myelinated outgrowth, typically ending in a growth cone (Fig. 1*a*). Sprouts may be nodal, preterminal or ultraterminal depending upon whether they take origin from a node, from the preterminal region, or from the terminals themselves. In the descriptions that follow 'sprouts' will be used to refer to nodal sprouts unless specified otherwise.

Sprouts are distinguished from *contributions*, which are defined as non-myelinated outgrowths from a node that terminate, not in growth cones, but in axon terminals on the same sole-plate as those of the parent ending (Fig. 2*b*). Sprouts and contributions are regarded as evidence of growth of the terminal axon (Barker & Ip, 1965, 1966) and will be referred to collectively as growth configurations. Growth con-

figurations typically arise from the last node of the terminal axon. The number of axon terminals formed by a contribution may be more or less than the number belonging to the parent ending. The different modes of origin and termination of growth configurations are illustrated in Figs. 10–16 and 25–27.

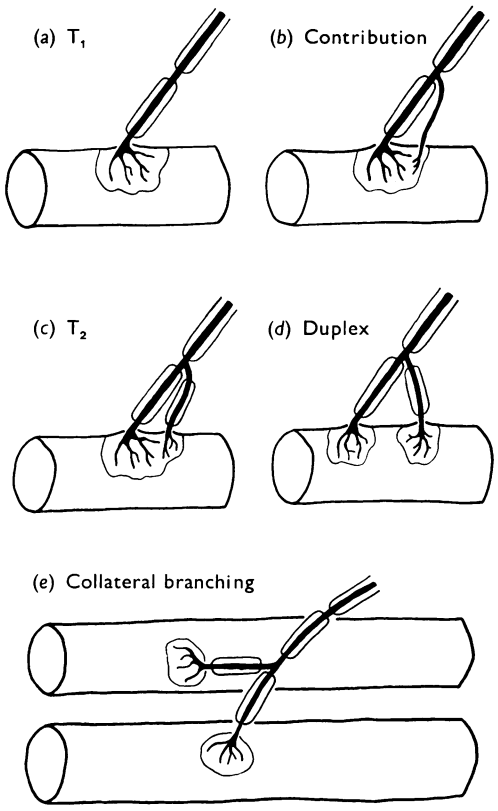

Fig. 2. Diagram to illustrate the terms used to describe morphological features of motor end-plates. The extent of the sole-plate of each ending is indicated. (*a*) Simple T_1 ending formed by an unbranched myelinated terminal axon. (*b*) T_1 end-plate bearing a contribution, i.e. a non-myelinated outgrowth from a node forming axon terminals on the sole-plate of the parent ending. (*c*) T_2 end-plate formed by two myelinated branches of the terminal axon, which both end on the same sole-plate. (*d*) A terminal axon divides and the axon terminals of the two branches lie on separate sole-plates forming a duplex ending. (*e*) Collateral branching. A terminal axon divides, giving two collateral end-plates on separate muscle fibres.

All cases in which a terminal axon supplied motor end-plates to more than one muscle fibre were carefully recorded. Such instances involved the *collateral* branching of the terminal axon (Fig. 2*e*). The search for such branching was extended as far proximally as possible, frequently into the intramuscular nerve trunks. Examples of the collateral branching of terminal axons are illustrated in Figs. 10 and 32. The total amount of collateral branching in a muscle is expressed by the Functional Terminal Innervation Ratio (F.T.I.R.), i.e. the ratio of the number of muscle fibres

innervated to the number of terminal axons (Edds, 1950; Coërs, 1955; Coërs & Woolf, 1959).

End-plates resembling configurations seen after nerve section were encountered in normal muscles. These were considered to be undergoing degeneration and were classified as in early, middle or late degeneration, according to the comparable configurations after nerve section. The morphological changes of end-plates after nerve section have been briefly described by Barker *et al.* (1970). Examples of all three phases of degeneration after nerve section are illustrated in Figs. 19–21 and comparable configurations from normal muscles in Figs. 17 and 18.

The replacement of distal axons by axonal debris was never observed in unoperated muscles. However, end-plates whose axon terminals showed fragmentation were occasionally encountered, and these were classified as showing late degeneration.

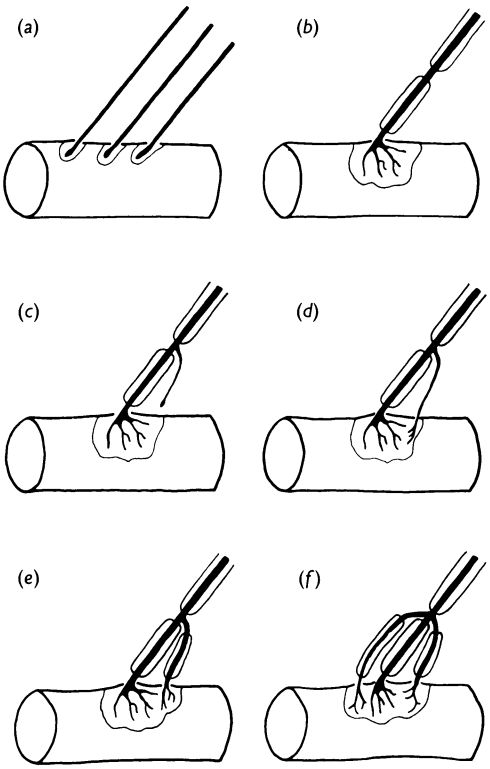

Fig. 3. The elaboration of motor end-plates during development and ageing. (*a*) Early in development the muscle fibres receive multiple innervation (see Redfern, 1970). (*b*) Later this is reduced to a single end-plate, typically of the T_1 form, on each muscle fibre. (*c*) A nodal sprout grows towards the parent ending and (*d*) contributes axon terminals to it. (*e*) Subsequent myelination of the contribution leads to the formation of a T_2 end-plate. (*f*) Further elaboration in this way leads to the formation of more complex end-plates.

Morphology of the motor end-plates of young adult cats

The main results of the examination of motor end-plates of PDQ and soleus muscles from animals of various ages are summarized in Table 1. In addition, the differences between end-plate types in terms of their growth and degeneration will be described.

Peroneus digiti quinti

The motor end-plates of the young adult PDQ have well-developed, fine axon terminals, usually ending in tapers, though knobs and rings also occur. In a range of end-plate types, T_1 end-plates occur about four times as often as T_2 end-plates (77·0 % and 21·5 % of the sample, respectively). One T_4 end-plate was observed. No duplex end-plates were found in the muscles included in the sample, although they were encountered in other PDQ muscles.

All the growth configurations were associated with the sole-plate of the parent ending. Their endings sometimes lay among the axon terminals of the parent ending, but were more often located closely adjacent to them. This was seen particularly clearly when the sole-plate nuclei were well stained (Figs. 11–16).

Growth configurations were associated with 15 % of all end-plates; contributions occurred with twice the frequency of nodal sprouts (Table 1). Proportionately more growth configurations were associated with T_1 end-plates than with T_2 end-plates, due to the relatively high frequency of T_1 end-plates receiving contributions (11·4 % compared with 5·6 % of T_2 end-plates). The proportions of sprouts associated with these two types of end-plate were approximately equal. Less than 1 % of end-plates received more than one growth configuration; in each case there were two growth configurations of the same type (i.e. either nodal sprouts or contributions). Occasionally, a contribution with a more or less marked thickening of its axon was encountered. Such configurations are interpreted as indicating that myelination is taking place (Fig. 16).

The incidence of collateral branching was extremely low (F.T.I.R. = 1·02), equivalent to 43 terminal axons supplying motor end-plates to two separate muscle fibres. A single terminal axon was never seen to innervate more than two muscle fibres and no degenerating collateral end-plates were encountered. No cases were seen of a sprout passing to a muscle fibre other than the one bearing the parent ending, i.e. new collateral end-plates were not being formed. The proportions of T_1 and T_2 collateral end-plates and the incidence of growth configurations were not significantly different from the corresponding figures for the sample as a whole. Although no T_3 collateral end-plates were found in any of the three muscles included in the sample, a single instance of a terminal axon branching to form two T_3 end-plates was encountered in another PDQ muscle.

Of the 14·8 % of degenerating motor end-plates in PDQ muscles of young adult cats, 70 % were in the early phase of degeneration. Approximately equal proportions of T_1 and T_2 end-plates were affected, but none of the 35 T_3 end-plates (1·5 % of the sample) showed any sign of degeneration. Growth configurations were associated with 30 % of all degenerating end-plates (i.e. 5 % of the sample). In such cases the

axon terminals of the contribution had the same retracted or swollen appearance as those of the parent ending. In other words, where a growth configuration occurred on a degenerating end-plate, it too was affected by the degeneration process.

Soleus

When compared with those of PDQ, the motor end-plates of the young adult soleus have a less delicate appearance. They are smaller and the terminal axon is frequently curled so that the axon terminals lie to one side of it, as noted by Nyström (1968*a*). The axon terminals themselves are fewer, shorter and coarser and often end in knobs rather than the tapers or rings common in PDQ.

Table 1. *Results of the morphological examination of motor end-plates of peroneus digiti quinti (PDQ) and soleus muscles of cats of various ages*

(The individual animals are identified and their ages and body weights are given. The values for the frequencies of the principal types of end-plate and of growth and degeneration are expressed as percentages of the total number of end-plates (*n*) in each sample.)

	Young adult PDQ (C254, C230) 2·80 kg (*n* = 2339)	10 year old PDQ (C294) 4·40 kg (*n* = 757)	Young adult soleus (C254) 2·80 kg (*n* = 614)	6 year old soleus 5·53 kg (*n* = 119)	10 year old soleus (C294) 4·40 kg (*n* = 500)	18 year old soleus (C296) 2·25 kg (*n* = 500)
T_1	77·0	45·1	99·0	59·7	87·6	73·8
T_2	21·5	47·4	1·0	36·1	12·0	24·4
T_3	1·5	7·0	Absent	4·2	0·2	1·4
Nodal sprouts	5·6	24·8	2·0	9·2	7·4	12·8
Contributions	10·0	16·7	2·1	7·6	12·0	19·8
Total growth	15·6	41·5	4·1	16·8	19·4	32·6
Early degeneration	10·3	Absent	4·2	—	1·0	—
Middle degeneration	4·4	1·6	7·3	—	2·4	—
Late degeneration	0·1	Absent	0·5	—	1·4	—
Total degeneration	14·8	1·6	12·2	—	4·8	—
F.T.I.R.	1·02	1·03	1·02	1·02	1·01	1·01

Soleus end-plates are less complex than those of PDQ. Thus, the proportion of T_2 end-plates is only 1 % compared to 21·5 % in the young adult PDQ, and the more complex T_3 endings are completely absent (Table 1).

Although there is much less evidence of growth in the soleus than in PDQ, all the growth configurations are in contact with the sole-plate of the parent ending, as in the latter muscle. Only one end-plate with more than one growth configuration was encountered.

Collateral motor end-plates occurred with the same frequency as in PDQ, and all were T_1 end-plates, as would be expected by the predominance of these endings in the sample. Only one collateral end-plate showed evidence of growth. One pair of collateral end-plates was in the middle phase of degeneration.

The assessment of degeneration in soleus muscles is more difficult than in PDQ because of the coarser appearance of the endings, but the swollen, retracted axon terminals of middle degeneration are generally unmistakable on careful scrutiny

(e.g. Figs. 28 and 29). Degenerating end-plates occur with about the same frequency in soleus and PDQ (12·2 % and 14·8 % of the samples, respectively), but in soleus there is a predominance of end-plates in the middle phase of degeneration. Growth configurations were associated with eight (10·3 %) degenerating end-plates in soleus (Table 1).

Morphology of the motor end-plates of old cats

The results of the morphological study of the end-plates of muscles from young adult cats confirm Barker & Ip's (1966) observations on the growth and degeneration of normal end-plates. However, they do not support their hypothesis of end-plate 'replacement' for two reasons. Firstly, all the growth configurations were in contact with the sole-plate of the parent ending, and there was no evidence of the formation of new end-plates by substitution (Fig. 1*d-f*) or collateral sprouting. Secondly, if 'replacement' were taking place, it would be expected that a majority of degenerating end-plates would receive a growth configuration. In fact, only 30 % of all degenerating end-plates in PDQ do so, and the proportion in soleus is even lower (10·3 %). Consequently, the 'replacement hypothesis' is untenable. If the alternative hypothesis of end-plate elaboration (Fig. 3) is valid, then it would be expected that the proportion of more complex end-plates would be greater in the muscles of older animals.

Ten year old peroneus digiti quinti

The most striking feature of the terminal innervation of these muscles was the marked increase in the proportion of the more complex end-plates. T_2 end-plates formed 47·4 % of all endings compared with 21·5 % in the young adult PDQ muscles (Table 1). There was also an increase in the proportion of T_3 and T_4 end-plates and a few T_5 end-plates were also present.

The incidence of growth configurations was three times as high as in the young adult (41·5 % and 15·6 % respectively). Contributions were less frequent than sprouts, unlike the situation in the young adult muscles, where they were the more frequent type of growth configuration. Ultraterminal sprouts were occasionally observed (Fig. 34). The proportions of T_1 and T_2 end-plates receiving growth configurations were in the ratio of 2:1, although the ratio of sprouts to contributions was the same for both types of end-plate. The values for T_3 end-plates must be treated with caution because of the relatively small sample size (53), but they showed less evidence of growth than either of the two commoner types of motor end-plate. The ratio of sprouts to contributions was the same as that for the commoner types of ending. The incidence of end-plates receiving more than one growth configuration was 3·6 %, a marked increase over the young adult muscles. As in the young adult muscles, configurations interpreted as myelinating contributions occurred (Fig. 27).

Collateral branching occurred with about the same frequency as in the young adult muscle, there being only a difference of 0·01 in the F.T.I.R., which is not significant (Coërs & Woolf, 1959). One terminal axon branched to form three motor end-plates on separate muscle fibres. All the other cases of collateral branching resulted in the innervation of two muscle fibres by one terminal axon. No degenerating collateral end-plates were observed. There were 18 collateral T_2 end-plates (37 % of all collateral end-plates), fewer than expected from the proportion of these endings in the whole

sample (47·4 %). Similarly, there were proportionately fewer growth configurations (20 % compared with 41·5 % in the whole sample).

The proportion of degenerating end-plates was lower than in the young adult, only eight degenerating end-plates being observed. All of these were in the middle phase of degeneration, and only one received a growth configuration (i.e. 0·2 % of the total sample).

The motor end-plates of this older animal were often of bizarre appearance with somewhat elongated and even coiled axon terminals. The terminal nerve fibres were often folded up on themselves and in some instances even appeared to overlie the axon terminals. Vesicular axonic swellings were occasionally present on axon terminals, nodal sprouts, or on nerve fibres in the intramuscular nerve trunks (Figs. 31–34). Such swellings have been described by Barker *et al.* (1970) on the non-myelinated parts of cat γ fusimotor fibres, and by Coërs & Woolf (1959) and Harriman, Taverner & Woolf (1970) in various normal and pathological human limb muscles. The sole-plate nuclei were generally well stained and appeared to be more frequent than in the young adult, as noted by Gutmann & Hanzlíková (1965) in senile rats (compare Figs. 13–14 with Figs. 25–27). Selected examples of motor end-plates from the muscles of older cats are illustrated in Figs. 25–30, 32 and 34.

Soleus muscles of 6, 10 and 18 year old cats

The changes in the morphology of motor end-plates associated with ageing resemble those in PDQ. The increase in the incidence of the more complex endings and growth configurations is even more striking than in PDQ because of the paucity of these configurations in the young adult (Table 1). Although there is considerable variation in the actual values for the proportions of the more complex endings in these animals, the increase is unequivocal when compared with the corresponding values for the young adult.

The end-plates of all three older cats bear a much higher proportion of growth configurations than those of the young adult. About 90 % of these were associated with T_1 end-plates. The 10 year old animal, alone among the older animals, shows a preponderance of nodal sprouts over contributions. In this muscle a nodal sprout was seen passing to a muscle fibre other than the one bearing the parent ending, the only example observed. However, it ended in a vesicular swelling and did not appear to make contact with the muscle fibre, which was itself supplied with a separate end-plate.

A few motor end-plates from the older animals bore ultraterminal sprouts, which were never encountered in young adult material. They were quite short and never passed to a muscle fibre other than the one innervated by the parent ending. Only one end-plate from the 10 year old cat received more than one growth configuration, the same number as in the young adult. The proportions of such configurations in the 6 and 18 year old animals were much greater (2·5 % and 2·2 % respectively). Some contributions were apparently undergoing myelination, as observed in PDQ.

The F.T.I.R. of all the older soleus muscles is the same or slightly lower than in the young adult. Only one collateral end-plate (in the 6 year old cat) was of the T_2 form; all the others were T_1 end-plates. One example of a terminal axon branching to

supply motor end-plates to more than two muscle fibres occurred. Only two collateral end-plates from these muscles showed evidence of growth.

The 10 year old cat was the only animal in which it was possible to assess the proportion of degenerating motor end-plates, because the end-plates of the others had undergone some post-mortem autolysis rendering it impossible to see the fine detail of the axon terminals. The incidence of degenerating endings in this muscle is very low relative to that of the young adult (4·8 % and 12·2 % of the samples respectively), as is the case in PDQ. As in the young adult there is a preponderance of endings in the middle phase of degeneration. Equal proportions of T_1 and T_2 endings were affected by degeneration. Growth configurations were associated with 2 (8·3 %) of the 24 degenerating end-plates (i.e. 0·4 % of the sample). Both were nodal sprouts and the end-plates were in the final phase of degeneration.

Table 2. *Results of measurements of end-plates and muscle fibres of PDQ muscles*

(The data are shown for each form of the end-plate, arranged in order of increasing complexity. The sample means are the values for the largest possible samples and not necessarily derived only from the data shown. In each group *a* represents the mean number of axon terminals per end-plate, *b* the mean end-plate diameter (μm), and *c* the mean muscle fibre diameter (μm). The number of end-plates in each group (*n*) and the standard error of the mean (*s.e.*) are given. The probability (*P*) of significant differences between corresponding values of muscles of different ages is given; non-significant differences are indicated by N.S. Significant deviations from the normal distribution are indicated by superscripts (see text).)

		Young adult PDQ				10 year old PDQ		
End-plate type		*n*	Mean	(*s.e.*)	*P*	*n*	Mean	(*s.e.*)
T_1	*a*	129	5·6	0·16*	<0·001	46	4·5	0·22
	b	65	26·6	0·61	<0·05	7	23·9	1·16*
	c	65	53·0	1·29	<0·05	7	59·0	2·20
T_1+sprout	*a*	24	7·6	0·38	<0·001	22	5·2	0·42†
	b	18	26·3	0·93	N.S.	8	25·9	1·77
	c	18	53·1	1·68	<0·02	8	65·7	4·86
T_1+contribution	*a*	40	8·2	0·30	<0·001	22	6·0	0·39
	b	28	28·6	1·15*	N.S.	7	26·6	1·01
	c	28	53·9	1·21	<0·01	7	67·5	4·06
T_2	*a*	51	7·9	0·36	<0·001	85	4·9	0·15‡
	b	28	29·4	1·14	N.S.	17	29·3	1·54
	c	28	53·5	1·72	<0·001	17	67·3	3·08
T_2+sprout	*a*	4	10·3	0·75	<0·001	17	6·0	0·47
	b	5	37·9	2·62	<0·05	4	29·1	2·95
	c	5	64·3	3·56	N.S.	4	64·1	9·05
T_2+contribution	*a*	5	12·6	1·90	<0·001	2	8·5	0·50
	b	6	37·2	4·81	—	—	—	—
	c	6	57·4	3·32	—	—	—	—
T_3	*a*	6	8·2	0·40	N.S.	7	6·6	0·69
	b	7	30·1	2·16	N.S.	4	31·0	3·84
	c	7	53·9	4·89	N.S.	4	69·5	8·06
Sample mean	*a*	197	6·3	0·14‡	<0·001	229	5·3	0·12‡
	b	171	28·7	0·49†	N.S.	50	27·4	0·78†
	c	171	53·7	0·72	<0·001	50	65·5	1·70‡

* g_1 and g_2 statistically significant; † g_2 statistically significant; ‡ g_1 statistically significant.

Measurements of end-plates and muscle fibres

A study of measurements, made in the same way, under similar conditions and by the same operator, may shed light on the relationships of end-plates and muscle fibres and show whether these relationships are changed in older animals.

The results of the measurements of motor end-plates, their muscle fibres and the number of axon terminals are summarized in Tables 2 and 3. In both tables the data are presented separately for each stage in the elaboration process in order of increasing complexity (i.e. simple T_1 end-plates first, then T_1 end-plates receiving nodal sprouts and so on up to T_3 endings). Even where the sample sizes are small the values have been shown for the sake of comparison. The mean values for each muscle are included, but must be considered with caution because the samples were not all randomly taken; the rarer forms of end-plate were often specifically sought in order to increase sample sizes. The sample sizes (n) for the end-plate and muscle fibre diameters are the same for each end-plate type, but the estimates of the number of axon terminals were made on larger numbers of end-plates. Statistically significant differences between each of the three parameters in muscles from cats of different ages are indicated, and also significant deviations from the normal distribution.

The results of this analysis demonstrate two principal points. Firstly, within each muscle there is a tendency for more complex end-plates to have more axon terminals and to lie on larger muscle fibres. Secondly, the mean muscle fibre diameter increases, and the mean number of axon terminals decreases, with ageing. The end-plate diameters are unaffected by these changes.

Peroneus digiti quinti

In both young and old animals there is a tendency for the number of axon terminals to increase with increasing complexity of the end-plate. This is to be expected according to the elaboration hypothesis proposed above. Thus, in the young adult with the addition of a sprout to a simple T_1 end-plate, the mean number of axon terminals rises from 5·6 to 7·6. A further increase (to 8·2) occurs when the sprout becomes a contribution by forming axon terminals. The mean muscle fibre diameter shows the same tendency to increase with increasing complexity of the end-plate, but the end-plate diameter shows no significant change.

The mean muscle fibre diameter is significantly greater in the older animal for each form of the end-plate (except where the sample size is small). Thus the sample mean of the 10 year old PDQ is 65·5 μm compared with 53·7 μm in the young adult muscle. The increase in the muscle fibre diameters is reflected in their distributions, that of the older animal being shifted to the right relative to that of the young adult, and showing a moderate skew, with a second smaller peak at the upper end of the range (Fig. 4).

The end-plate diameter is not significantly greater in the older animal, and there is therefore a significant decrease in the percentage ratio of end-plate and muscle fibre diameters ($P < 0{\cdot}001$). The mean number of axon terminals is significantly decreased in the older animal. In both samples the muscle fibre and end-plate diameters are significantly correlated, but the dispersion is considerable (young adult, $r = 0{\cdot}37$, $P < 0{\cdot}001$; 10 year old, $r = 0{\cdot}35$, $P < 0{\cdot}02$). It was only possible to

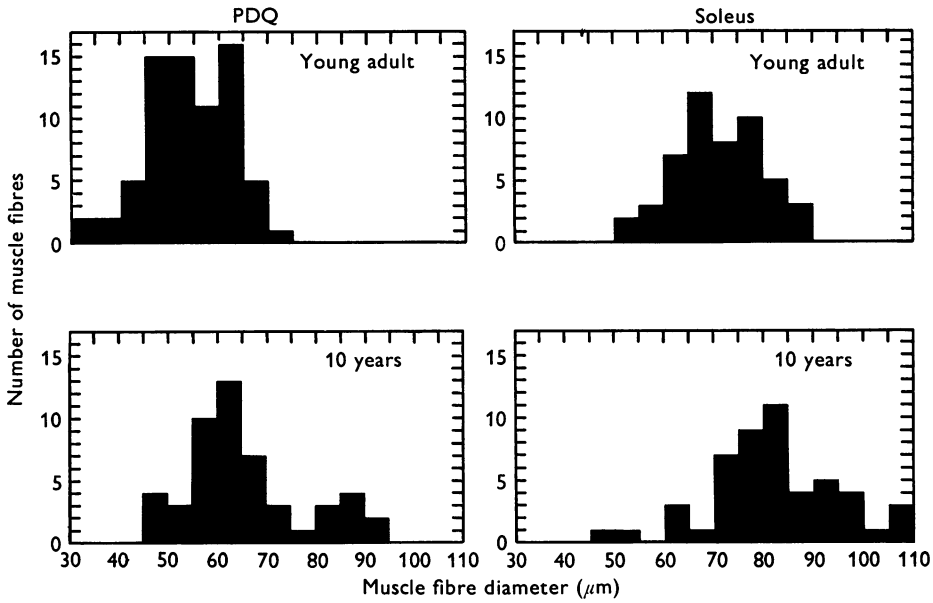

Fig. 4. Histograms of muscle fibre diameters of PDQ and soleus muscles of young adult and 10 year old cats. The number of fibres measured is 72 in the young adult PDQ, 50 in each of the other muscles. The mean values are: young adult PDQ, 56·7 (*s.e.* 1·40); young adult soleus, 70·9 (*s.e.* 1·21); 10 year old PDQ, 65·6 (*s.e.* 1·70); 10 year old soleus, 81·4 (*s.e.* 1·78). The distribution in the PDQ of the 10 year old cat (lower left) shows a slight positive skew ($g_1 = 0{\cdot}77$, $P < 0{\cdot}05$). The other three distributions are normal. All the histograms are plotted on the same co-ordinates to facilitate comparisons.

Table 3. *Results of measurements of end-plates and muscle fibres of soleus muscles. For description see legend to Table 2*

		Young adult soleus				10 year old soleus		
End-plate type		*n*	Mean	(*s.e.*)	*P*	*n*	Mean	(*s.e.*)
T_1	*a*	129	4·3	0·12	<0·01	82	3·7	0·16*
	b	41	22·7	0·82†	N.S.	33	25·3	1·08
	c	41	71·4	1·25	<0·001	33	82·5	2·40
T_1+sprout	*a*	5	5·8	1·46	N.S.	7	3·6	0·30
	b	—	—	—	—	3	15·7	2·75
	c	—	—	—	—	3	81·9	5·90
T_1+contribution	*a*	4	4·5	0·50	N.S.	10	4·4	0·22
	b	—	—	—	—	10	26·3	1·72
	c	—	—	—	—	10	76·5	3·07
T_2	*a*	—	—	—	—	9	5·0	0·41
	b	—	—	—	—	3	26·5	3·56
	c	—	—	—	—	3	88·4	3·19
Sample mean	*a*	139	4·4	0·13	<0·01	108	3·9	0·14*
	b	41	22·7	0·82	N.S.	50	24·8	0·90
	c	41	71·4	1·25	<0·001	50	81·4	1·78

* g_1 statistically significant; † g_1 and g_2 statistically significant.

correlate the numbers of axon terminals with the other parameters in the young adult animal and, in this case, there was some correlation ($n = 72$, $r = 0{\cdot}28$, $P < 0{\cdot}02$). The mean value of the axial ratio is 1·15 in the 10 year old animal, significantly lower than in the young adult ($P < 0{\cdot}05$). This indicates that the end-plates become more rounded with ageing.

Soleus

Because of the paucity of complex end-plates and growth configurations in the young adult muscle (Table 1) it was not possible to obtain sufficient numbers to merit statistical analysis of these forms and they are shown in Table 3 solely for comparison. Therefore the results will be described without reference to the different forms of the end-plate, although in the 10 year old cat there is a tendency for the number of axon terminals and muscle fibre diameter to increase with increasing complexity of the end-plate (Table 3).

As in PDQ, there is a marked increase in the mean muscle fibre diameter with increasing age (young adult, 71·4 μm; 10 year old, 81·4 μm; $P < 0{\cdot}001$), reflected in the distributions of the measurements (Table 3 and Fig. 4). Similarly, the decline in the mean number of axon terminals with ageing is also present, and the mean end-plate diameters are unaltered. The mean axial ratio is unchanged in these muscles, indicating that the shape of the end-plates remains constant. The end-plate and muscle fibre diameters are not significantly correlated in soleus muscles.

On comparing the results for the young adult PDQ muscles with those of a soleus muscle from the same animal it is seen that the values for all 3 parameters shown in Table 3 show highly significant differences ($P < 0{\cdot}001$). Soleus has a greater mean muscle fibre diameter, but the mean number of axon terminals and the mean end-plate diameter are lower, reflecting the differences in the morphology of the end-plates of these muscles. The mean axial ratio is the same in these muscles, indicating that the end-plates are of the same shape.

Muscle and body weights

The means of the weights of the two PDQ and soleus muscles of each of nine cats were calculated and plotted against their body weights (Fig. 5). There is a highly significant degree of correlation between the muscle weight and body weight in both cases. Such a result would certainly be expected in soleus, which is primarily a postural muscle, but not necessarily in the 'phasic' PDQ. The body weights of the 6, 10, 15 and 18 year old cats were 5·53, 4·4, 2·6 and 2·25 kg, respectively. This indicates that the body weight tends to decline from middle age, so that it is lower in the senile animals, as found by Rowe (1969*a*) in the rat.

Number of muscle fibres

The cat is not a suitable animal for quantitative studies of ageing phenomena, because of the difficulty of obtaining sufficient numbers of older individuals to ensure adequate statistical samples. However, counts of the numbers of muscle fibres present in transverse sections through the belly of PDQ muscles of aged cats did indicate that muscle fibres are lost with increasing age. Thus, the numbers of muscle fibres in PDQ muscles of cats aged 1, 18 and 19 years were 4,794, 2,409 and 1,852

respectively. This finding confirms the observations of several workers on various muscles of the rat (Andrew, Shock, Barrows & Yiengst, 1959; Gutmann & Hanzlíková, 1965; Rowe, 1969*a*).

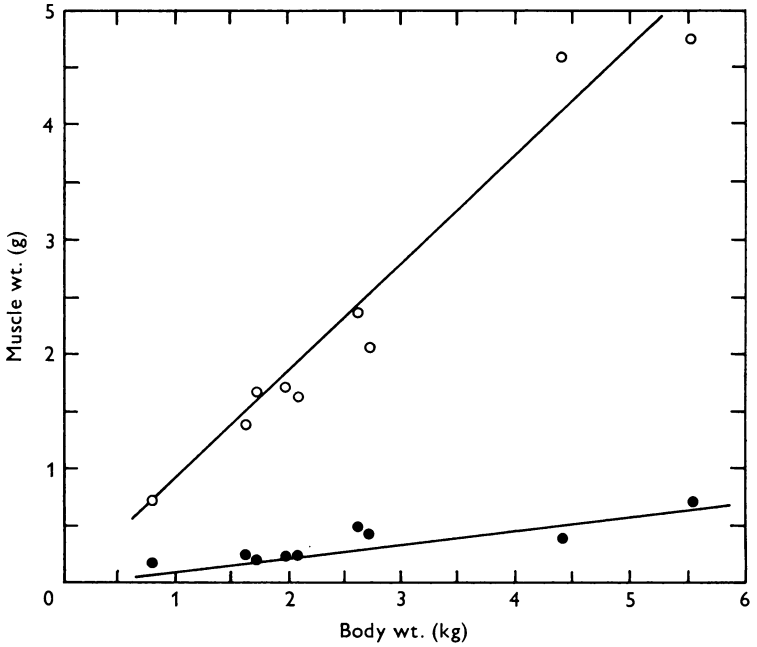

Fig. 5. Graph of muscle weights (g) of PDQ (filled circles) and soleus (open circles) against body weights (kg) of 9 cats aged 15 days to 19 years. The mean values of both muscles from each animal are plotted. The correlations for both muscles are highly significant ($P < 0{\cdot}001$).

DISCUSSION

In a morphological study based upon a single staining method, it is important to consider what proportion of the observations might be due to artefacts. The features most likely to be attributed to this cause are non-myelinated outgrowths from the terminal axon and degenerative changes of the axon terminals. Apart from the rigorous selection of well-stained areas for examination, other factors militate against this possibility. Firstly, end-plates showing growth or degeneration are scattered throughout the muscle in terminal sprays containing normal simple end-plates (Figs. 23 and 24). Secondly, the variations between animals and muscles are consistent. Thus, the end-plates of PDQ muscles of different young adult cats are similar in all respects, and conversely the end-plates of a soleus muscle differ markedly from those of the PDQ of the same young adult animal. The changes occurring with age are also consistent. Finally, many of the changes observed in older animals have been observed by other workers using silver and methylene blue staining methods on rat and human muscles (Gutmann & Hanzlíková, 1965; Harriman *et al.*, 1970).

Consequently it must be concluded that Barker & Ip's (1965, 1966) finding of end-plates undergoing growth and degeneration in normal hind limb muscles is

confirmed. The results will be discussed in relation to their hypothesis of 'end-plate replacement', before considering the alternative of elaboration (Fig. 3). Growth and degeneration will be discussed separately.

Growth of motor end-plates

Relatively few degenerating end-plates also received a growth configuration, as would be expected if they were undergoing replacement by rejuvenation or substitution (Fig. 1). Further, in every case where a contribution was associated with a degenerating end-plate, it too was affected by the degeneration process.

The fact that all the growth configurations observed were in contact with the sole-plate of the parent ending excluded the possibility of the formation of new end-plates by substitution (Fig. 1 *d–f*) or collateral sprouting.

The incidence of collateral branching, as shown by the F.T.I.R. (Table 1), was very low (1·01–1·03) in all the muscles examined. This compares with a value of 1·10 reported for various normal rat and human muscles (Edds, 1950; Coërs, 1955; Coërs & Woolf, 1959). This low level of collateral branching is not considered as evidence of collateral re-innervation for three reasons. Firstly, no evidence of any new collateral outgrowths was found. Secondly, cases of collateral branching were found in the muscles of a 10 week old kitten, and Coërs (1955) noted that the F.T.I.R. values of infants were the same as those of adults. It is therefore reasonable to conclude that collateral branching is part of the normal pattern of innervation. Finally, if collateral re-innervation were occurring it would be expected that the F.T.I.R. values would be greatly increased in the muscles of older animals. Such is clearly not the case (Table 1), indicating that the replacement of end-plates by collateral outgrowths does not occur.

The early workers on collateral re-innervation of partially denervated muscles considered that sprouting was stimulated by the products of degeneration of nerves and muscles (Edds, 1953). The absence of collateral re-innervation in normal muscles may be because the incidence of degeneration is too low to provide the necessary stimulus. Certainly the proportion of degeneration in experimental and pathological muscles where collateral re-innervation occurs is always greater than the 15 % found in normal muscles of young adult cats.

There have been few physiological studies of motor units in normal human subjects

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Figs. 6–10. Different types of motor end-plates from normal young adult PDQ muscles.

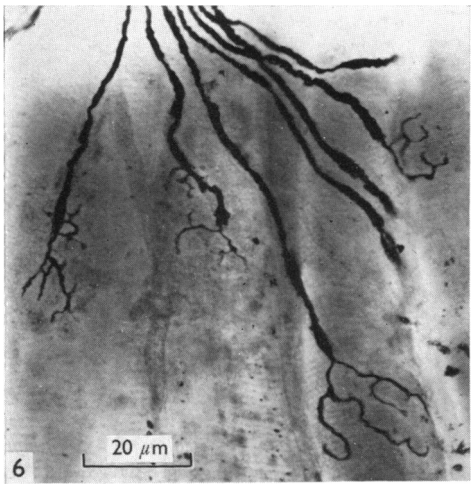
Fig. 6. Part of a terminal spray of T_1 end-plates. The motor end-plate second from the left has a small contribution. The axon terminals are delicate and typically end in fine tapers. The sole-plate nuclei are lightly stained.

Fig. 7. T_2 end-plate. The terminal axon bifurcates at the penultimate node and the branches supply unequal numbers of axon terminals. The two groups of axon terminals are closely adjacent.

Fig. 8. T_3 end-plate. All three myelinated branches of the terminal axon arise from the same node and supply approximately equal numbers of axon terminals.

Fig. 9. T_4 end-plate. The terminal axon branches at two different nodes to form four myelinated branches, whose axon terminals are intermingled.

Fig. 10. Collateral branching occurs at a node (*n*). One branch forms a T_2 end-plate (far right), the other a T_1 end-plate (far left) which receives a nodal sprout (*n.sp.*).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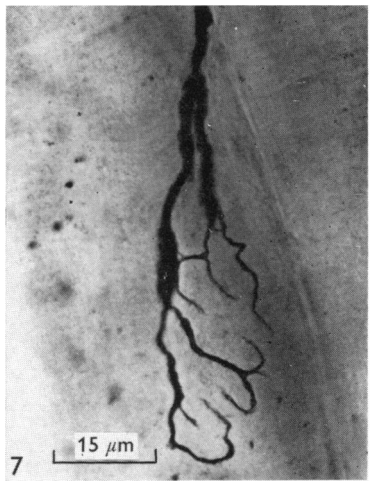
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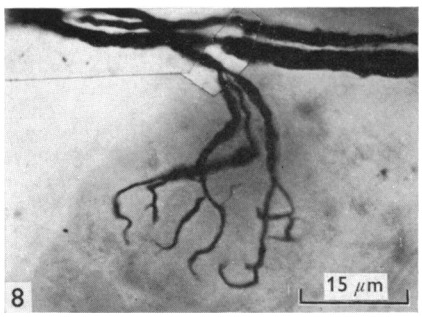
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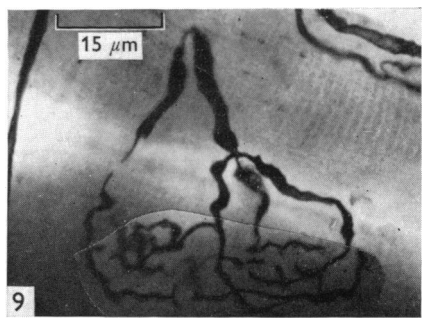
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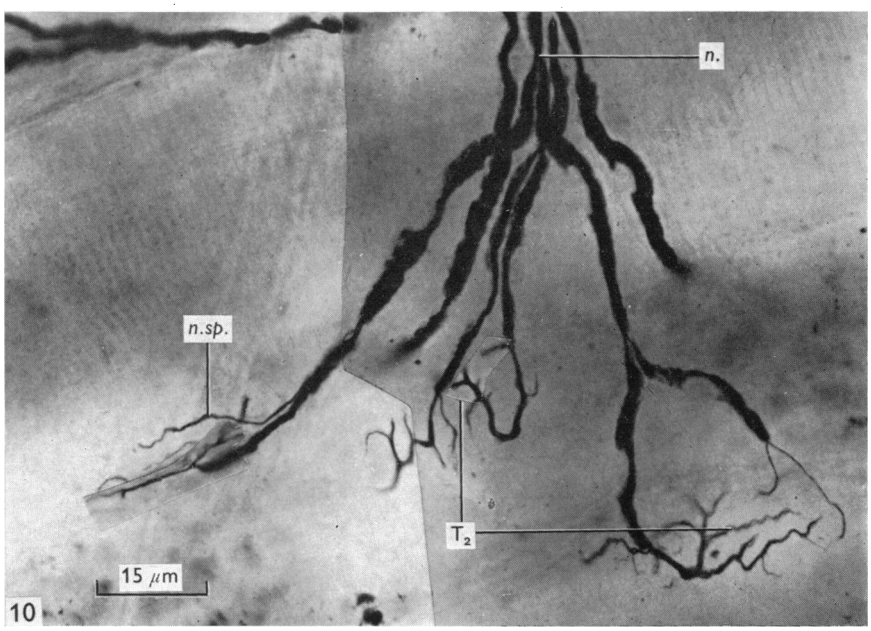
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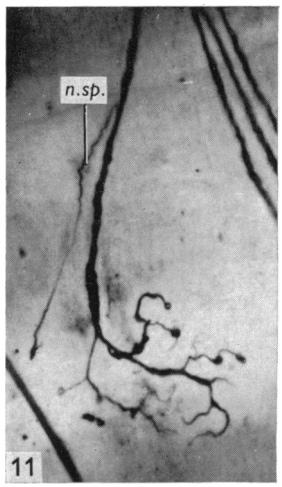
n.sp.
T2
15 μm
10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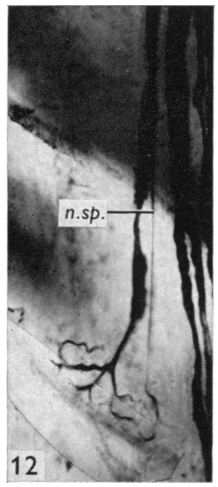
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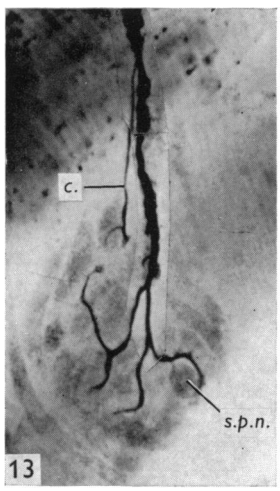
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s.p.n.
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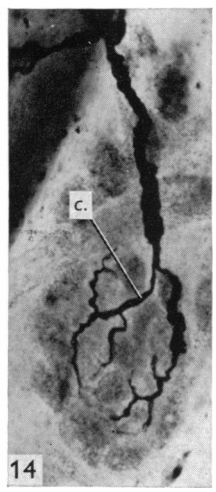
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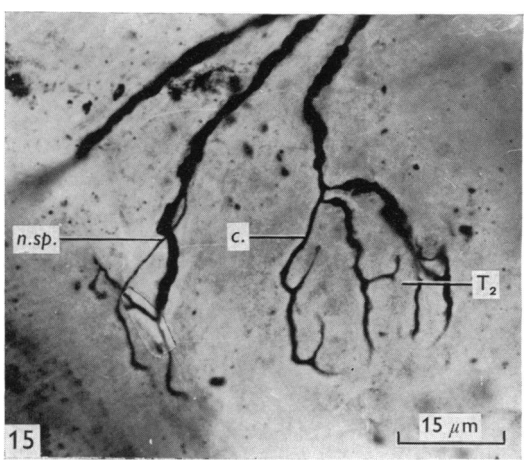
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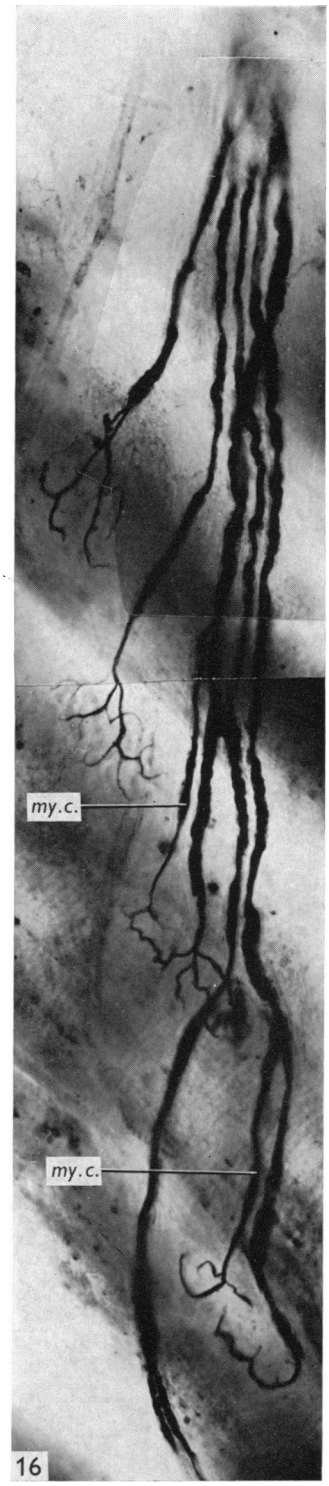
15


my.c.
my.c.
16

after the sixth decade, but such studies have indicated that the mean size of motor units is increased (Sacco, Buchthal & Rosenfalck, 1962; McComas, personal communication), which could be interpreted as evidence of collateral re-innervation. However, Sacco *et al.* suggest that the changes in the characteristics of motor units are caused by alterations in the topography of end-plates and muscle fibres (probably due to differential growth and atrophy). Campbell & McComas (1970) have shown that there are fewer motor units in older subjects, and it is therefore possible that the increased mean size of motor units may be due to the preferential loss of small motor units, rather than to collateral re-innervation.

It is evident therefore that although growth and degeneration occur with approximately equal frequency in the muscles of young adult cats, they are not causally related as supposed by Barker & Ip (1965, 1966). More important is the fact that the replacement hypothesis does not account for the increase in the proportions of the more complex end-plates associated with ageing. Thus, according to the replacement concept, after the addition of a new myelinated branch to the end-plate the original branch would degenerate, replacing one simple T_1 end-plate by another, and there would be no net increase in the proportion of more complex endings.

Growth and ageing

The incidence of growth configurations rises dramatically with ageing from 4·1 % in the young adult soleus to 32·6 % in the senile 18 year old cat (Table 1). It is probable that some of the growth configurations in older animals do not form functional synaptic contacts with muscle fibres. This is supported by the increased occurrence of end-plates receiving more than one growth configuration, and the presence of vesicular axonic swellings in the muscles of the older cats. Barker *et al.* (1970) have suggested that axonic swellings on axon terminals and sprouts are indicative of the inability to form functional synaptic contacts. Other workers have suggested that a high incidence of growth configurations, presence of axonic swellings, and the elaboration of end-plates are 'manifestations of ageing of the lower motor neurone' (Harriman *et al.*, 1970, p. 402).

Occasionally, a large ramifying end-plate whose fine axon terminals formed

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Figs. 11–16. Growth configurations from young adult peroneal muscles. All to the same scale.

Fig. 11. T_1 end-plate receiving a nodal sprout (*n.sp.*) which arises from the penultimate node and ends in a growth cone close to the axon terminals of the parent ending.

Fig. 12. The nodal sprout (*n.sp.*) arises from the penultimate node and ends very close to the axon terminals of the parent ending.

Figs. 13–14. T_1 end-plates receiving contributions (*c.*). The sole-plate nuclei (*s.p.n.*) are stained, clearly showing that the contributions terminate on the sole-plate of the parent ending. In Fig. 14 the contribution arises from the terminal node.

Fig. 15. A T_1 end-plate (left) receives a nodal sprout (*n.sp.*) which arises from the terminal node and ends among the axon terminals of the parent ending. A T_2 end-plate (right) receives a contribution (*c.*).

Fig. 16. Terminal spray of motor end-plates. The two lower end-plates receive contributions with thickened axons (*my.c.*) considered to indicate that myelination is occurring. The two upper end-plates are simple T_1 endings.

spirals around the muscle fibres was encountered in the soleus of the 18 year old cat. Such enlarged end-plates have been described in cases of dystrophia myotonica, and attributed in part to the 'hypertrophy of muscle fibres which characterizes the condition' (Coërs & Woolf, 1959; Allen, Johnson & Woolf, 1969, p. 24). The striking increase in the diameters of muscle fibres in older animals has already been noted (Tables 2 and 3).

The tendency for the proportion of sprouts to rise relative to the proportion of contributions in older animals may be due to their slower maturation. Moyer, Monafo & Kaliszewski (1960) found that there was no difference in the rate of nerve growth in cats of different ages, but there are no data on rates of myelination in animals of different ages.

One result of ageing is that the end-plates of soleus come to resemble those of PDQ, in terms of the incidence of growth and complex forms. This could be another aspect of the diminution of the differences between fast and slow muscles that occurs with ageing (Drahota & Gutmann, 1963).

Degeneration of motor end-plates

If, as concluded above, degenerating end-plates are not replaced, it seems most likely that they are lost and that their muscle fibres eventually atrophy and disappear. This is supported by the reduced number of muscle fibres in the PDQ muscles from the older cats. Similar results have been obtained in muscles from senile rats (Andrew *et al.*, 1959; Gutmann & Hanzlíková, 1965; Rowe, 1969*a*; Tuffery, unpublished). However, no evidence of muscle fibre atrophy was found in transverse sections of muscles from cats of various ages. Nor were there any small-diameter muscle fibres in the samples measured in teased silver preparations (Fig. 4).

The degeneration of motor end-plates was ascribed by Barker & Ip (1966) to the loss of nerve cells described by Duncan (1934) and Gardner (1940). Certainly the fact that degenerating end-plates are found scattered throughout the muscle suggests

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Figs. 17–24. Degenerating motor end-plates from young PDQ muscles. All to the same scale.

Fig. 17. Degenerating end-plate from a normal muscle. The axon terminals have local thickenings, typical of the early phase of degeneration (cf. Fig. 19).

Fig. 18. Degenerating end-plate from a normal muscle. The axon terminals have a swollen, slightly retracted appearance characteristic of the middle phase of degeneration (cf. Fig. 20).

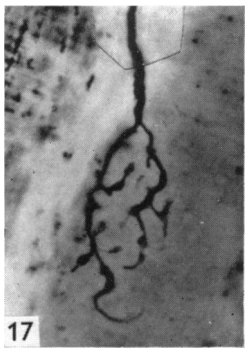
Figs. 19–20. Configurations comparable to Figs. 17–18 seen 24 h after section of the muscle nerve 1 cm from muscle entry.

Fig. 21. The end-plate (*d.pl.*) in the centre is completely broken down and the distal axons are replaced by axonal debris (*ax.d.*). That the changes after nerve section are not synchronous in all end-plates is shown by the intact end-plate on the left (54 h after nerve section).

Fig. 22. A T_2 end-plate with slightly inflated axon terminals has a vesicular axonic swelling (*v.a.s.*) on an axon terminal. The terminal axon bifurcates at a relatively proximal node. (Unoperated.)

Fig. 23. A T_1 end-plate (*d.*T_1) in early degeneration adjacent to a normal T_1 end-plate (T_1). (Unoperated.)

Fig. 24. A T_2 end-plate in early degeneration (*d.*T_2) occurs in a terminal spray containing two normal end-plates, one of which (T_2) is of the T_2 form. The sole-plate nuclei (*s.p.n.*) are stained, showing that the T_2 end-plates each have but a single sole-plate. A capillary (*cap.*) is also slightly stained. (Unoperated.)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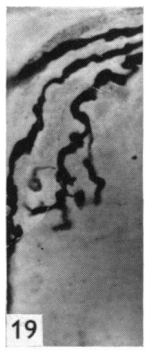
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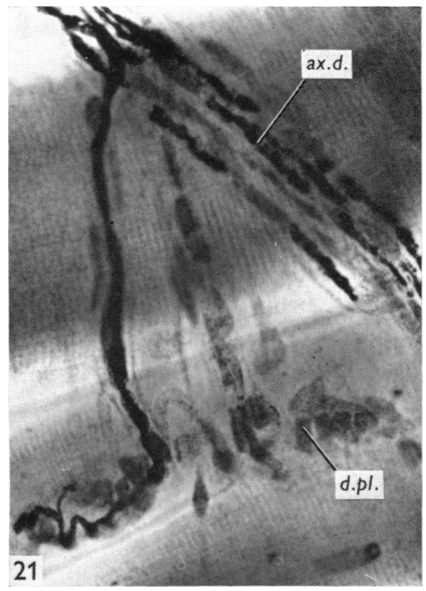
ax.d.
d.pl.
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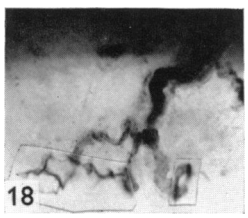
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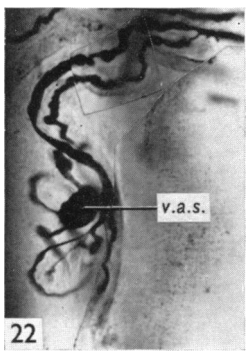
v.a.s.
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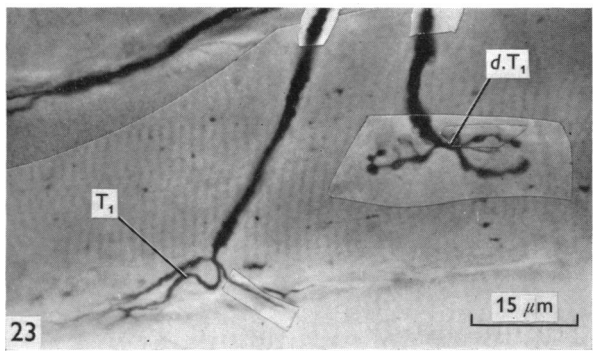
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T1
15 μm
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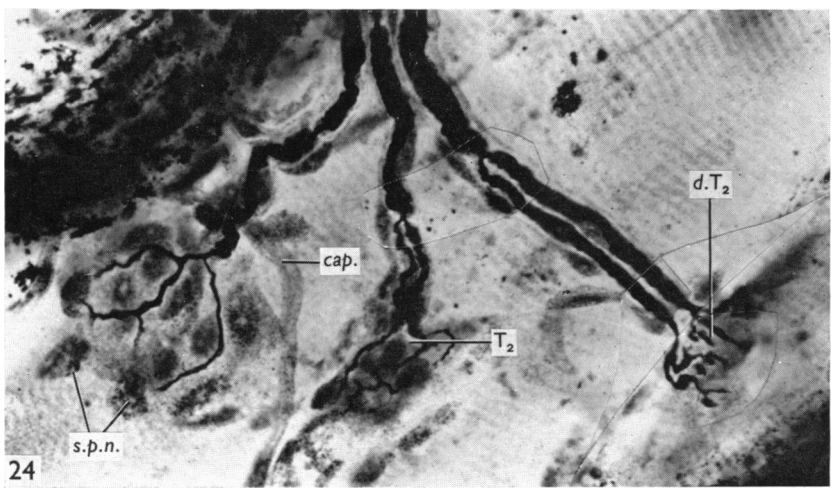
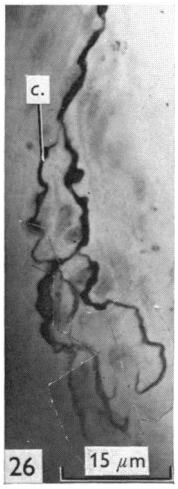
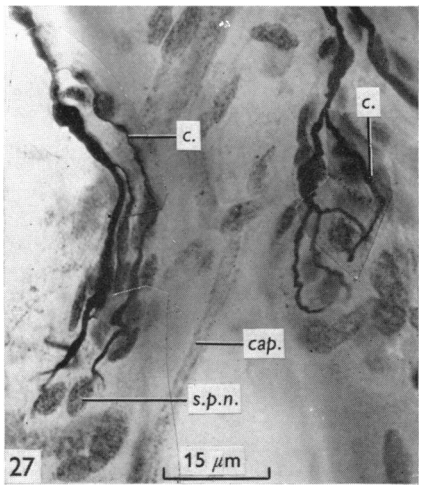

d.T2
cap.
T2
s.p.n.
24


n.sp.
10 μm
25


c.
15 μm
26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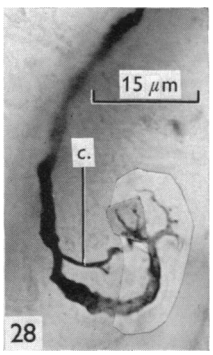
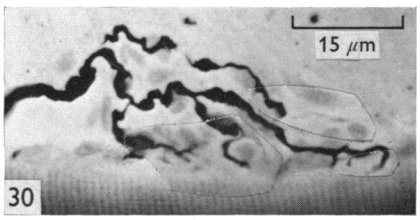
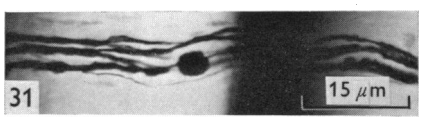
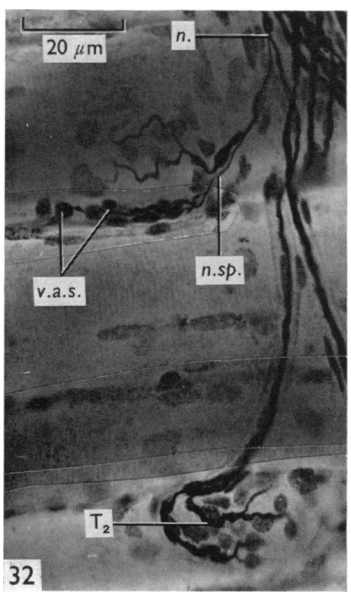
c.
cap.
s.p.n.
15 μm
27

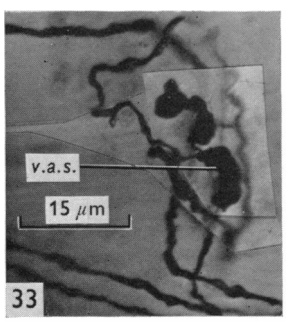

15 μm
c.
28


15 μm
29


15 μm
30


31
15 μm


20 μm
n.
n.sp.
v.a.s.
T2
32


v.a.s.
15 μm
33

u.sp.
v.a.s.
15 μm
34

a nervous origin (Gutmann & Hanzlíková, 1965), but they were unable to show a decrease in the number of α nerve fibres in the muscle nerves of senile rats. Moyer & Kaliszewski (1958) observed that it was 'not uncommon' to find degenerating fibres in the ventral roots of apparently healthy cats, but they were unable to demonstrate that there were significantly fewer nerve fibres in the ventral roots of aged cats (Moyer & Kaliszewski, 1958; Moyer, 1959).

From the observations of Henneman and his associates on the triceps surae muscles of the cat, it is clear that the loss of a very small number of motoneurones would suffice to account for the degenerating end-plates encountered since each may form over 500 end-plates (McPhedran, Wuerker & Henneman, 1965; Wuerker, McPhedran & Henneman, 1965). It is possible that the individual variation in the number of fibres is high enough to mask small decrements.

Gutmann, Hanzlíková & Jakoubek (1968) have suggested that, even in the absence of a demonstrable loss of motoneurones, the deleterious changes in the end-plates and muscle fibres may be due to their impaired metabolism and a decline in the trophic function of the motoneurone.

Elaboration of motor end-plates

The hypothesis that the growth configurations observed in the terminal innervation of unoperated muscles are part of a process of end-plate elaboration (Fig. 3) has been formulated in the light of the results of this investigation. It takes account of

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Figs. 25–34. Motor end-plates and terminal axons from PDQ and soleus muscles of a 10 year old cat (C294).

Fig. 25. T_1 end-plate with few axon terminals receiving a nodal sprout (*n.sp.*), which arises from the terminal node. The sole-plate nuclei are stained, showing that the sprout ends on the sole-plate of the parent ending. Note the large number of sole-plate nuclei. PDQ.

Fig. 26. T_2 ending receiving a contribution (*c.*) with very slight thickening proximally. The terminals of the contribution are among those of the parent ending. PDQ.

Fig. 27. Two end-plates receive contributions (*c.*) which show thickening of their axons, considered to indicate myelination. The end-plate to the left has two contributions, one from each of the last two nodes. To the right, the axon terminals of the contribution are among those of the parent ending. Sole-plate nuclei (*s.p.n.*) and capillaries (*cap.*) are stained. PDQ.

Fig. 28. A T_1 end-plate in the middle phase of degeneration. The terminals of the contribution (*c.*) are also swollen. PDQ.

Fig. 29. Soleus end-plate with the inflated axon terminals typical of middle degeneration.

Fig. 30. T_4 end-plate in profile. The axon terminals of the four branches are in close proximity. PDQ.

Fig. 31. Part of a small intramuscular nerve trunk showing a vesicular axonic swelling on a nerve fibre. PDQ.

Fig. 32. A terminal axon branches at a node (*n.*) giving rise to two end-plates. The upper end-plate is of the T_1 form and receives a bifurcating nodal sprout (*n.sp.*) which ends in two vesicular axonic swellings (*v.a.s.*). The lower collateral end-plate is of the T_2 form. The two branches of the terminal axon forming this end-plate clearly end on the same sole-plate. PDQ.

Fig. 33. Vesicular axonic swelling (*v.a.s.*) on a coiled terminal axon just distal to an intramuscular nerve trunk. This axon eventually formed a normal T_1 end-plate. PDQ.

Fig. 34. A T_2 end-plate bears an ultraterminal sprout (*u.sp.*) which ends in a double vesicular axonic swelling (*v.a.s.*), a short distance from the parent ending. PDQ.

the fact that all the growth configurations are in contact with the parent end-plate, and that there is a marked increase in incidence of complex end-plates in the muscles of older animals. This view is further supported by the existence of configurations which indicate that myelination of contributions occurs (Figs. 16, 27).

The concept of elaboration can usefully be applied to the changes in end-plate morphology in the neonatal and young animal, as well as to the period from adulthood to senescence. Thus, it is known that the end-plates of immature animals are very small, often consisting of a single axon terminal (Coërs, 1955; Nyström, 1968*a*). According to Nyström (1968*a*), the end-plates of the cat are becoming fully differentiated at about 10 weeks, and in the present study end-plates of the T_2 form were observed at this time.

An explanation for the process of end-plate elaboration may be found by considering the work load imposed upon the neuromuscular system. Clearly in the kitten there is a great increase in work load as the animal becomes larger and more active. At the same time the size of the end-plates and the number of axon terminals increase and the area of synaptic contact is enlarged.

In order to attribute the increase in the proportion of complex end-plates in older animals to an increasing work load it is necessary to demonstrate that the work load does in fact increase throughout adulthood.

The data on body weight suggest that it continues to rise until senile wasting sets in at about 15 years. This results in an increasing load upon the muscle fibres that is exaggerated by the loss of muscle fibres due to the degeneration of end-plates. With the onset of senility, it might be expected that the rate of loss of muscle fibres would be accelerated, thus maintaining the load on the remainder.

That the muscle fibres respond to the increasing work load is evident from the correlation between muscle weight and body weight (Fig. 5), and the fact that most of the increase in muscle weight caused by overloading is due to an increase in the contractile component of the muscle (Rowe, 1969*b*). This is reflected in the change in the distribution of the muscle fibre diameters, resulting in a larger mean diameter (Fig. 4; Rowe, 1969*a*) as occurs after surgically induced hypertrophy (Rowe & Goldspink, 1968) and exercise (Goldspink, 1964). Further work is in progress to determine the effects of increasing the work load upon the morphology of the end-plates of the young adult cat.

That the work load could be the prime factor in causing the observed increase in the frequency of growth configurations and complex end-plates is indicated by the results for soleus muscles in Table 1. If allowance is made for senile wasting in the 18 year old cat, it can be seen that there is a good relationship between these two parameters and body weight.

Other workers have reported hypertrophied muscle fibres in elderly human subjects, and Rubinstein (1960) suggests that such fibres compensate for the atrophy and loss of other muscle fibres. Serratrice, Roux & Aquaron (1968) also reported muscle fibre splitting, a phenomenon which has been associated with increasing demands upon the muscle fibre (Edgerton, 1969; Hall-Craggs, 1970; Rowe & Goldspink, 1968).

It appears then that the work load imposed upon the neuromuscular system does increase throughout life and that the muscle fibres respond to the increasing demands

made upon them. However, since the end-plates of older animals are not larger than those of the young adult, and have significantly fewer axon terminals (Tables 2 and 3), the size of the end-plate is not the controlling factor. It is suggested that it is in fact the number of myelinated branches of the terminal axon forming the end-plate which is the important feature.

The effect of increasing the number of myelinated branches constituting an end-plate will be to increase the number of nerve impulses arriving at that end-plate, since each branch will propagate and conduct the impulse separately. This would mean that a greater proportion of the area of the neuromuscular junction would be affected, thus compensating for the decrease in synaptic area relative to the size of the muscle fibre (Tables 2 and 3), as well as any decline in the trophic function of the motoneurone.

End-plate types, muscle fibre types and motor units

The results of the present study confirm those of Nyström (1968*b*) in showing that the diameter of a fibre is an unreliable guide as to its histochemical type. Consequently, although there is a correlation between end-plate type and muscle fibre diameter, it is not possible to deduce any relationship between it and muscle fibre type. The present study also confirms Nyström's (1968*b*) observation that the soleus muscle fibres of the cat are all of the same histochemical type, which is not identical to any of the types found in 'mixed' muscles. Therefore no valid inferences about the relationships of end-plate types to muscle fibre types in other muscles can be made from the cat soleus.

From the disposition of degenerating end-plates in the muscle, it would be expected that the growth and degeneration of end-plates is controlled by the motoneurone. It was hoped that collateral pairs of end-plates, as examples of endings in the same motor unit, would elucidate this point. Unfortunately, because of the very low incidence of collateral branching in the muscles studied, it was not possible to obtain sufficient examples to indicate what the nature of the control of growth and degeneration might be. Certainly it was not consistently found that both members of a pair of end-plates derived from the same terminal axon showed evidence of growth, or were of the same form (i.e. T_1 or T_2). If growth is controlled by the motoneurone, the observations show that the process is not simultaneous in all the 'daughter' end-plates.

Finally, consistent marked variations occur in the incidence of growth and degeneration of motor end-plates, as well as in their size and form. These variations are related both to the specific muscle examined and to the age of the animal from which it is taken. Therefore, if erroneous conclusions about possible pathological changes in the morphology of end-plates are to be avoided, it is essential to study adequate samples of end-plates from normal specimens of the test muscle.

SUMMARY

A quantitative examination of silver-impregnated peroneus digiti quinti (PDQ) and soleus muscles of cats aged 1–18 years confirms the existence of growth and degeneration of motor end-plates in normal muscles. When end-plates are classified according to the number of myelinated branches of the terminal axon forming them,

a clear and consistent variation is observed between PDQ and soleus, and between young and old cats. The incidence of complex end-plates is greater in PDQ, but in both muscles complexity increases with age. The incidence of collateral branching remains constant at 2 %. The muscle fibre diameter is significantly increased in older animals. Bizarre end-plates and axonic swellings were found in the muscles of older animals. It is considered that growth and degeneration are not causally related and that degeneration leads to loss of muscle fibres. Growth is seen as part of the process of 'end-plate elaboration', and considered to be an attempt to increase the use of the area of synaptic contact in response to (*a*) an increasing work load in growth and adulthood, and (*b*) a decline in the trophic function of the neurone and increasing loss of muscle fibres in senility. These findings are discussed in relation to other studies on the effects of ageing and activity on the neuromuscular systems of man and animals.

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