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Meat Studies No. 1.—Post-natal Growth and Development of Muscle, as Exemplified by the Gastrocnemius and Psoas Muscles of the Rabbit.

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CONTENTS.

CHAPTER 1.

* In preparing for publication minor changes have been made in the text. An additional chapter on relative growth by D. van der Reyden, Section of Statistics, has been included.

CHAPTER I.—INTRODUCTION.

(a) INTRODUCTION.

HITHERTO the subject of meat production was approached mainly from the feeding side, and generally stopped at the digestion of the foodstuff and the body weight of the animal.

This traditional approach was broken by Hammond (1932). He made observations on the final product—meat—and worked backwards to determine the conditions and factors which affect its formation. Macroscopic methods mainly, were applied in making a general survey of the scientific principles involved in the production of meat, from the physiological, anatomical and practical points of view.

Scattered throughout the literature are isolated references to microscopical meat studies, on small numbers of animals, of different species and varying uniformity. Due to the diversity of conditions under which the observations were often made, and in many cases too, due to a lack of accurate definition of procedure, these studies cannot easily be co-ordinated.

A wide field of investigation lies open to the worker who approaches the problem of meat quality from the histological point of view. Definition of the quantitative character of muscle, in terms of measurable biological entities such as muscle bundle and muscle fibre, constitutes a primary requisite for such an investigation. In addition, the qualitative changes occurring in meat must be considered in relation to the variations in the morphology of muscle.

Such a microscopic biological study will establish a basis for studying meat in the various domestic animals. It will facilitate evaluation of various factors which affect its formation. Furthermore, that elusive character, meat quality, may be brought a stage nearer to precise determination when considered in terms of such study.

Accordingly, this work is devoted to a study of the morphological changes of muscle and its component units, during growth and development. The object is a general survey of the principles involved in muscle growth, particularly as it occurs within the individual muscle. It is hoped that these observations may suggest profitable lines of experimental work, dealing with development of muscle and meat quality.

Although it would be of advantage to commence investigations on the domestic animals used for meat production, such observations would be costly and time-consuming. Preliminary observations on an animal species completing its life cycle in a short while yield information at less expense and in a shorter period of time. Such information may be of value in establishing various factors concerning growth of muscle and its development.

Small laboratory animals live under different conditions from the usual meat animals. Moreover, the general principles of growth may not be identical in small and large domestic animals. Nevertheless, the information obtained may serve a useful purpose, by making it easier to plan meat investigations.

Preliminary observations indicated that the rabbit was more suitable than the other laboratory animals for the purpose of this study. Hence rabbit muscle was utilised for this work.

(*b*) Object of Work.

The contractile properties of voluntary muscle have been investigated almost exclusively in cold blooded animals such as the frog, mainly because such muscle may be isolated and kept alive for a considerable time. Study has largely been confined to a few muscles such as Gastrocnemius, Soleus, and Sartorius. The physiology of warmblooded mammalian muscle is, to a large extent, interpreted in terms of the experimental behaviour of such frog muscle. This is not without difficulty. It is hardly surprising when the wide range of mammalian muscle, of varying architecture and function, is taken into consideration. Moreover, the work has been concerned with physiology (the nature of muscular contraction, its chemistry, and its efficiency), rather than morphology as such. Obviously there is still a wide field open for experimental investigation.

On the other hand, the long series of researches by Hammond (1932), and his co-workers Pálsson (1939-40), Verges (1939a, 1939b), and McMeekan (1940-41), represents ,, a return to the practice of the older days when animal physiology was not yet divorced from morphology ''. These authors have dealt with the differential growth of constituent parts of the body in terms of muscle, fat, and bone, with the object of clarifying the biological problems involved in meat production. Muscle has been considered in terms of its proportional development in the different parts of the animal body. As the economic value of meat depends primarily on the proportion of edible meat to inedible parts of the carcass, these workers have utilised weight as a basis for their investigations.

However, growth and development of muscle mass must ultimately depend on growth and development initiated in the micro-structure of the muscle. Muscle is conceived as a network of connective tissue, binding together a mass of fibres which form the greater part of a muscle. Morphological change of these muscle fibres must largely determine the change in morphology of the gross muscle, as well as its change in weight.

In the present work, the morphology of muscle has been studied, with the object of deciding how this changes during growth and development. This is necessarily the first step. The next step is to determine what growth and developmental changes occur in the individual muscle fibre, and whether they account for the coincident change in size and shape of the muscle mass. Special emphasis will be laid on the relation between the growth made during successive periods, with the object of deciding whether further research on these lines is likely to prove profitable.

On account of the nature of the investigation, statistical treatment of the data collected is essential in order to obtain reliable quantitative results. However, the laborious character of the microscopic measurement in work of this nature places severe limitations on the extent of work which can be undertaken. This is likely to limit the value of the results by reason of the restricted scope of the investigation.

Hammond (1932) showed, that if muscles are arranged in different anatomical groups, growth follows well-defined gradients. However, individual muscles within the different groups " vary in their rate of growth and overlap in many places those of other groups ". It follows that the behaviour of the muscle group as a whole cannot be accurately assessed from any single muscle within the group.

It can be inferred that similar difficulties are inherent in the present study, if attention is confined to a limited number of muscles. There is the danger fallacious properties may be attributed to musculature in general. Furthermore, although a localised description of isolated muscles may show up minor variations it is not likely to afford any idea of the general laws of growth. Justification of the method lies in the fact that the present investigation is only a preliminary step to decide whether morphological analysis of muscle growth is likely to prove a profitable avenue of meat research. Subsequently the study may be expanded to include muscular tissue throughout the animal body, in order to establish more closely the relationship between muscle growth and development, and muscle type and structure.

Morphological study of muscle growth may appear remote from the basic problem in mind, namely meat investigation. It is to be emphasised that knowledge regarding the structural composition of muscle affords a ready means of comparison of meat, not only from different muscles within the same carcass, but also from different carcasses of varying grade and quality.

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CHAPTER II.—REVIEW OF LITERATURE.

For convenience the literature will be discussed under headings corresponding with those employed in the treatment of the experimental data. First, however, it is appropriate at this stage to consider briefly growth and relative growth.

By means of his autocatalytic theory of growth Robertson (1923) holds that master reactions regulate growth cycles, represented by peaks in the growth curve. However, Robb (1929) discredits this theory, as the cycles may be explained by natal and juvenile growth retardations, inevitably associated with the disturbance of birth, and of endocrine re-organisation at a later stage of life. Snell (1929) points out an inherent defect in the theory that growth rate is controlled by autocatalytic processes. MacDowell, Gates, and MacDowell (1930) also do not favour this interpretation. Hammond (1932), and Walton and Hammond (1938) disagree with the autocatalytic theory as they show that the growth curve is dependent on the food supply; thus, " these peaks at regular times in the life of an animal are due to the nutritive conditions usually existing at these times ".

Recently analysis of growth has become increasingly directed towards the mathematical generalisation of experimental data. General formulae have been devised for describing the growth of the organism as a whole. Although these theoretical expressions are useful for comparison and tabulation, there is danger in attempting to define the fundamental growth process itself from empirical formulae.

In order to understand the growth of an organism it is essential to analyse the changes in form of the organism. Huxley (1924, 1932), and Huxley and Teissier (1936), formulated a law of simple allometry which is applicable over long periods of the animal's life, after completion of the stage of histological differentiation. When the growth of a part is considered in relation to the rest of the body, the relative rate of growth of the part and of the body remains constant. Huxley's equation expresses this law of relative growth by means of a formula $y = bx^a$, where y equals the

part, x the whole, b a constant representing the value of y when x equals 1, and a the equilibrium constant of the part. When a is greater or less than unity, the part is growing more or less rapidly respectively than the whole, that is, positive or negative allometry.

For a wide variety of data, this equation has been fitted to express the relation between a part and the whole, as the organism increases in size. Cursory inspection of the literature discloses an amazing diversity of interests. [Pearsall (1927), Keys (1928), Robb (1929), Hersh (1931, 1934, 1938), Green and Fekete (1933), Needham (1932, 1934), Dawes and Huxley (1934), Lerner (1936), Rytand (1937-38), Gray and Newcombe (1938), Hamilton and Dewar (1938), Clark and Hersh (1939), Huggins (1940), Crozier (1940), Brody (1942), Kibler, Bergman and Turner (1943), Richards and Kavanagh (1943)]. The list is by no means complete, and it is intentionally selective in order to emphasise the general manner in which Huxley's equation has been applied.

There is no doubt that the formula affords a useful method of comparing curves of growth. Thus, instead of only being able to present a record of the differences of absolute size with age, the formula makes it possible to disclose more clearly the underlying morphological changes by showing alterations in the proportions of individual parts with increasing total size. By means of the equilibrium constant a, a measure is obtained of the relative increase or decrease of the part with increase in the absolute size of the organism.

On the other hand, doubts exist as to whether biologists have been sufficiently critical regarding the application of the formula, and whether the fit of their data to this equation is real or not. Discussion has taken place regarding the implications of Huxley's formula, both from the viewpoint of possible shortcomings as well as its undoubted advantages [Robb (1929), Davenport (1934), Bernstein (1934), Wilson (1934), Feldstein and Hersh (1935), Richards (1936), Lumer (1936, 1939), Kavanagh and Richards (1942), Lumer, Andersen and Hersh (1942)].

Huxley makes it clear that the allometric law is limited to the growth occurring after the processes of histo-differentiation have been completed. Thus, the varying proportions of newly-born reciprocal crosses between the large Shire horse and the small Shetland pony are determined by genetic influences acting before birth (Walton and Hammond, 1938). Although the proportions of the body subsequent to birth follow the law of allometry, nevertheless the constant a is approximately the same for the different crosses. Similarly, Pontecorvo (1929, 1938) finds that the equilibrium constant a is very nearly the same for widely differing breeds of cattle. Notwithstanding this similarity in their relative growth rate, the different breeds vary greatly in the adult condition. Apparently the initial absolute size of the parts and of the body at birth must play a big rôle in determining body size and proportions in later life.

Huxley also demonstrates the presence of certain growth gradients where the values of the relative growth rate a, obtained for a series of parts arranged in order along the organism, change systematically from one end of the series to the other. Similarly, growth centres are found from which the growth intensity grades downwards, by comparing the parts of an organism on the assumption that the values of a are constant within each portion. Although it is reasonable to assume that the equilibrium constant

will vary in a progressive manner from point to point within the limits of a single part, the analysis does not take account of this continuous variation. Such an analysis is, however, likely to yield a more accurate knowledge of the growth mechanism.

(*a*) Growth and Development of the Rabbit.

Post-natal growth has been extensively studied for most animal species. Reference to Brody, Ragsdale and Elting (1926), or Hammond (1932, 1940a) gives an idea of the extent of literature available.

Absolute weight increases slowly in the growing animal at first, then more rapidly. The live-weight curve for rabbits normally shows a steady rise gradually flattening with age as maturity is approached. About the period of sexual maturity it receives a temporary check. Later, an increase in weight is again noticeable in most cases and the animal subsequently becomes somewhat heavier, largely due to deposition of fat after the cessation of growth (Punnett and Bailey, 1918; Castle, 1922; Pease, 1928).

While the animal is growing, body conformation and shape are undergoing continuous change as a result of the different parts growing at different rates (Hammond, 1932; McMeekan, 1940-41). Jackson and Lowrey (1912-13) point out that, in the rat, the intensity of growth passes over the body like a wave, reaching the maximum first in the head and fore-limbs, and later passing backwards along the trunk to the abdominal portion and hind legs. Hammond (1940a) states: " In general, the wave of growth beginning at the head, spreads down the trunk, and secondary waves which start at the extremities of the limbs pass upwards; these all meet at the junction of the loin with the last rib, which area is the last part to develop. Such growth gradients also exist between different tissues in the body which develop in the following order—brain, bone, muscle and fat."

Rate of growth varies in different breeds of rabbits. Large breeds usually mature more slowly than small breeds, hence, in general, the small rabbit will attain its mature weight earlier than a large rabbit (Dunlop and Hammond, 1937). Although heavy weight is closely associated with slowness of maturity, Pease (1928) points out that many rabbits show conspicuous absence of this association. Data have been presented showing a maximum rate of growth about 30 days after birth (Murray, 1921), sixty days (Wilson, 1930), whereas Dunlop and Hammond's (1937) large strains " E " and " H " attained the maximum about 100 days compared with 40 days in their small strain " F ". Robb (1929) finds a distinct tendency for two peaks in the lifetime of the animal, one about 40 days after birth and the other at about 100 days. These figures illustrate the differences inherent in different breeds.

It is hardly surprising that workers using different breeds in various parts of the world are not unanimous regarding the age at which the rabbit attains sexual maturity. Thus, Punnett and Bailey (1918) estimate puberty at 10 months in Polish and over 12 months in Flemish rabbits; Castle (1922) 6 to 7 months; Hammar (1932) 4 to 5 months; and Fangauf and Immenkamp (1938) 5 to 7 months.

Earlier workers regarded mature weight as the maximum weight attained during the first year of life. However, Pease (1928) points out that this arbitrary measure has no relation to the rate of growth of the rabbit. He uses instead, the " turning point ", in his comparative growth

studies. This is defined as the point where the live-weight curve for the individual rabbit slackens off at the oncome of puberty. Pease states the live-weight curve gradually rises again after the turning point, then declines, and finally rises once more to adult weight at about 400 to 500 days.

There seems to be little doubt that age is not as important as weight in influencing the normal body changes and proportions, as the magnitude of one body part tends to be a specific function of the total body mass (Robb, 1929; Huxley, 1932). In the albino rat, Outhouse and Mendel (1933) describe a close correlation between increase in weight and length. They found so little relationship to age, that body dimensions and proportions were identical in animals of the same weight irrespective of their age. Size of muscle, and organ too, is dependent on body size of animal, not age (Moment, 1933). Dunlop and Hammond (1937) show that changes in the body proportion of the rabbit occur with weight rather than with age as such. For sheep too, weight classes rather than age classes at shows are suggested by Hirzel (1939), because the proportion of muscle within the sheep's body is influenced by increase in weight more than age. It follows, therefore, in planning comparative growth studies, live-weight rather than age must form the basis of comparison of normal animals. It is to be noted, however, where growth is suppressed by under-nourishment, the magnitude of an organ or system may vary markedly for any given body-weight according to the age of the animal and the general state of nutrition (Jackson, 1932).

In most species, the male appears to be slightly heavier than the female at birth; in cattle (Hulce and Nevens, 1917; Eckles, 1920); in sheep (Donald and McLean, 1935; Phillips and Dawson, 1937; Bonsma, 1939); in pigs (Carmichael and Rice 1920; Murray, 1934); in guinea-pigs, Haines (1931); and in rats (King, 1935; Murray, 1941). As a rule the male continues to be heavier than the female, so that in most mammals the adult male is larger and heavier than the female. However, Kopec (1924) reports the weight of the two sexes is not essentially different in newly-born rabbits. Furthermore, Punnett and Bailey (1918) find the buck is in no case markedly heavier than the doe at maturity. Although the average weight is approximately equal in some cases, yet the doe is often markedly heavier than the buck. In the larger races of rabbits, the male has a bigger frame and is consistently larger in all bone measurements, nevertheless the female puts on more flesh and surpasses the male in weight (Castle, 1922). Castle cites breed standards for the various large breeds, in which rabbit breeders regularly specify a larger weight for females than for males. MacDowell (1914) is of opinion that the growth subsequent to four months of age is greater in the doe than in the buck. Pease (1928) could find no difference in average weight of the two sexes, but bodyweight was nearly always more variable in does than in bucks. He suggests a heavier weight is prescribed for does by show standards, because the female sex is more variable. This greater variability in live-weight of females is confirmed by Dudley and Wilson (1943). In addition, these authors find that the average live-weight of females after puberty is greater than that of males. Wilson (1930), and Wilson and Morris (1932), observed noticeable differences in the composition of male and female flesh. In general, the musculature of does at 11 months to 24 months of age contains 4 to 6 per cent. more fat than for the buck.

It is not clear how much the growth of rabbits is affected by the seasons of the year. Under the conditions of Pease's (1928) experiment, growth rate is unaffected by the season of the year in which the rabbit is born, or in

which it reaches maturity. However, Wilson (1929) holds that Spring and early Summer are the most favourable periods for satisfactory growth. This is contradicted by Bertelli (1936), who shows rabbits born during Autumn obtain their complete development sooner than those born in Spring.

(*b*) Growth and Development of Muscle.

1. *Weight.*

Jackson and Lowrey (1912-13) cite numerous authors regarding the relative weight of skeletal musculature in widely varying species. Thus, in most adult mammals, between 40 per cent. and 50 per cent. of the body-weight is composed of muscle. Among mammals, the smallest relative weights are found in the large animals, while the largest percentage of muscle is recorded in comparatively small animals [rabbit, 49·7 to 57·2 per cent., Weiske (1895); 49·4 to 56·3 per cent., Levine *et al* (1941).]

Hammar (1932) presents evidence that the musculature of the rabbit grows most rapidly, and has its period of greatest growth about puberty. He states muscle grows more than twice as much during the two months around puberty, as during the two months immediately preceding. Hence, the animal at puberty becomes muscularised to a striking degree.

Hammond (1932) and McMeekan (1940-41) show that, in the sheep and pig, bone makes its greatest growth in the early stages of life, followed by muscle at a later stage, while fat attains its maximum growth still later. In the rat, the skeletal musculature increases from a relative weight of 22·82 per cent. at one week old to 45·43 per cent. at one year of age, in sharp contrast to the skeleton which decreases from 18·47 per cent. to 10·91 per cent. (Jackson and Lowrey, 1912-13). In the fowl, the skeletal muscles increase from 21 or 22 per cent. at hatching to about 50 per cent. of the body weight in the adult, compared with a relative weight of the skeleton at hatching or slightly less than 16 per cent., afterwards decreasing to about 8 to 11 per cent. (Latimer, 1924). In the human, the relative weight of the skeleton remains practically unchanged from birth to maturity (17·69 and 17·60 per cent.), whereas voluntary muscle shows a marked increase from 24·80 per cent. in the newborn babe to 43·07 per cent. in the adult (Wilmer, 1940).

The increasing proportion of muscle with age is explained by Hammond (1932), by the greater rate of growth of muscle to bone in the different parts of the body, but also by the greater growth rate of the later maturing parts of the body which contain large proportions of muscle to bone (e.g. loin compared with head and limbs). Even after muscle has attained maximum development, there is an increase in inter- and intramuscular fat, which tends to increase the weight of muscle.

Hammond shows that weight of muscle, regarded as a measure, has a late period of maximum development, as it is an index of muscle and fat development. Length development is attained relatively early. Hence, because increasing muscle and fat development with age increase thickness of muscle, weight can also be considered as an indirect measure of muscle thickness.

Hammond's studies make it clear that muscle groups develop serially in a definite manner, corresponding to the differential growth gradients existing between the different parts of the body. Growth waves pass from lower to upper limb, from the cranium backward, and from the tail forward,

to meet in the lumbar vertebrae, so that muscle in the loin and pelvis makes the most growth post-natally. However, within each group of muscles the rate of growth of individual muscles varies greatly and overlapping occurs between groups. Hammond also shows how these normal age changes are emphasised by sex, breed, domestication, and fattening.

Hammond clearly indicates that if an individual muscle is to be taken as a sample of a carcass, it is advisable to select a muscle with a late rate of post-natal development. M. Psoas major has been studied as a physiological unit of musculature with a view to obtaining information on the musculature in general (Callow, 1935, 1936, 1937, 1938; Woodman, Evans, Callow and Wishart, 1936). This muscle has the advantage of relatively late development; moreover it can readily be removed without cutting the carcass. In the pig, McMeekan (1940-41) finds that the weight of the Psoas muscle has a significant correlation with the total weight of muscle in the carcass.

(2) *Length.*

By the time adult life is reached the long bones have achieved their maximum growth in length, the other tissues growing in proportion with the growth of the bones in length. Haines (1932) explains the growth in length of a muscle as following on the lengthening of the bones to which it is attached, in response to the traction set up within the muscle by the bone growth. This stretch, which the growing skeletal system places on the muscle, probably influences considerably the increasing strength of skeletal muscles in growing animals, as the period of greatest increase in strength coincides with the period of rapid increase in the length of the long bones (Knowlton and Hines, 1939). As muscle growth follows and is so closely dependent on bone growth, it is not out of place to digress for a moment to consider the growth of bone.

Hammond (1932) and McMeekan (1940-41) show that bone length reaches a maximum relatively early in life, before muscle development takes place. Hence, lengthening of any individual muscle must reach a maximum earlier than its growth in width and depth, which depend on the development of both muscular and fatty tissues.

With regard to the relative change in length of different muscles as the animal grows, the lengthening of muscle units in various parts of the body must be influenced by the well-defined differential growth relationship for individual bones, demonstrated by Hammond and McMeekan. In the heifer, Eckles and Swett (1918) report a greater degree of lengthening for the vetebral column than for the hind limb (117·3 to 66·3 per cent.). The same is true of the sheep. Hammond (1932) gives measurements for Suffolk rams, from which the following table has been calculated.

Relative length growth with age.

	Birth.	Three Months.	Five Months.	Four Years.
Lumbar vertebrae	100	305·6	330·0	427·8
Femur	100	214·8	247·9	309·9
Tibia	100	197·1	224·1	278·7
Cannon	100	176·5	187·9	213·6

Whereas the ruminant is born in a relatively mature condition with long legs to follow its dam, the rabbit is comparatively immature at birth. Hence, caution must be exercised in drawing an analogy between different species. However, for the pig, where the limb bones are not so well developed at birth compared with the sheep, the same tendency is shown by McMeekan (1940-41). The following table, compiled from his scale photographs of the lumbar vertebrae and femur, shows clearly that the lumbar vertebrae lengthen to a relatively greater degree than the femur.

Relative length growth with age.

	Birth.	Four Weeks.	Eight Weeks.	Twelve Weeks.	Sixteen Weeks.	Twenty Weeks.	Twenty-four Weeks.	Twenty-eight Weeks.
Lumbar vertebrae.....	100	154	231	292	339	423	431	477
Femur.....	100	154	217	253	287	329	345	370

It can be inferred that M. Psoas, which is closely adherent to the vertebral column, will show a similar difference in length growth, as compared with a muscle from the upper limb.

(3 *and* 4). *Width and depth.*

In general, width and thickness are late maturing body measurements (Bonsma, 1939). Latimer (1927, 1928) shows that, after puberty in the foal, there is no increase in the length of bone, but the bones become stouter and increase in weight. Similarly, in the rat, the adult bones are wider and thicker than at an earlier stage of development (Hammett, 1924). Also in the pig, thickness growth of bone is a late developing character compared with length of bone (McMeekan, 1940-41). It is shown by Hammond (1932), that in the sheep, growth in circumference of bone, i.e., thickness, persists after bone has ceased growing in length. This author demonstrates how the growth changes in muscle groups copy, in an exaggerated form, the coincident changes in the bones they surround. By analogy, it can be inferred muscle width and depth increase after length has become stabilised. As muscle width and thickness are an indirect measure of muscle and fat development, i.e., weight, which is a later maturing factor than length, this is to be expected.

Hammond (1936) observed the changes in shape of the Longissimus dorsi muscle, with increasing age of various species of domestic animals. He shows the medio-lateral axis (width) reaches maximum development earlier than the dorso-ventral axis (depth), so that depth of muscle becomes relatively greater in proportion to width of muscle, as an animal becomes older. McMeekan (1940-41) too, states that as the animal ages muscle width achieves stability, whereas depth increases at a still greater rate. A picture is presented of the muscle increasing equally in both width and thickness in the initial stages, later only by thickness growth in increasing amounts.

(*c*) Growth and Development of Muscle Bundle.

1. *Technique of measurement.*

Satisfactory demarcation of the bundle unit is an immediate difficulty in the morphological study of muscle.

Many workers have utilised a variety of methods to measure length of muscle bundle. This will be considered in connection with the muscle fibre (pages 341–342). Accordingly, they are not mentioned at this stage.

Although bundle length may be measured fairly easily, thickness of bundle is not capable of rigid definition. The smallest units, the primary bundles, are formed by a number of closely adjoining parallel muscle fibres held together by interstitial connective tissue. Several primary bundles combine to form secondary bundles, secondary bundles combine to form tertiary bundles, etc. A vast network of connective tissues binds together these bundles to constitute the individual muscle.

Hammond and Appleton (1932) judged bundle thickness by eye, because of the technical difficulties and labour involved in actual measurement. Sections were cut across the grain, from samples taken from the middle of the muscle. These sections were then graded, according to the coarseness of the component bundles. Hammond and Appleton point out that sectioning may introduce artefacts, as the bundles tend to fall apart more easily in some muscles than in others. Apart from this fact, in some muscles there are large bundles which are sub-divided into a number of smaller bundles, whereas in other muscles the bundles are all small. These authors confirm Piersol's (1920) finding that in muscles of coarse texture each bundle includes a number of sub-bundles, whereas in muscles with fine texture the secondary bundles correspond with the fasciculi.

Brady (1937), and Satorius and Child (1938) obtained a measure of bundle thickness, by counting the number of fibres in 50 bundles from each muscle, and by measuring the diameter of 50 muscle fibres from each muscle. McMeekan (1940-41) counted the fibres in 20 bundles selected at random, as well as measuring 100 fibres in each muscle.

2. *Thickness of muscle bundle* (*texture*, " *grain* ").

Texture is important mainly because coarse texture is associated with tough stringy meat [Hammond 1940(a), 1940(b), 1942]. However, Beard (1924) finds that " the inherent properties of the endomysium contribute to the toughness of meat more than does the size of the fibre ". Although there is also a broad correlation between toughness of meat and its connective tissue content (Mitchell and Hamilton, 1927-28; Moran and Smith, 1929; Mackintosh *et al*, 1936; Bate-Smith, 1942), observations by Hammond (Moran and Smith, 1929, page 42) show that the proportion of connective tissue to muscle substance is considerably higher in the tender meat of foetal lamb than in the tougher meat of an adult sheep. This finding is corroborated for the rat by Hines and Knowlton (1939). These authors calculate that the connective tissue decreases from 40 per cent. of the total muscle mass at 15 days to 15 per cent. at 90 days of age. Hirzel (1939) comes to the conclusion that " evidence on texture and connective tissue, their interrelation and the effect on toughness of meat is still scarce and inconclusive ".

Muscle texture is dependent on the size of the muscle bundles, which again depends on the number and size of the fibres comprising the bundle. Hammond and Appleton (1932) cite many authorities regarding texture of meat. Different muscles vary in texture; for example, Moran and Smith (1929) arrange beef muscles in order of increasing coarseness and toughness, from the M. Psoas (fillet), to Longissimus dorsi (rib), Biceps femoris (topside) and lastly Semimembranosus (silverside). Muscles are fine-grained at

birth, but corresponding with the degree of enlargement of the muscle fibres, so does texture become coarser as the animal becomes older. Hammond and Appleton (1932) are of opinion that where the fibres are small, texture does not coarsen with age as much as in large-fibred muscles. Apart from differences within the animal, species differences are also evident. In general, a large species (ox) has muscles with coarser texture than a small species (sheep). Hammond and his co-worker show that, within a species, similar differences are present between large breeds and the smaller breeds.

The niceties of gradation of texture largely remain to be worked out. The extremes are probably represented by bundles with small numbers of fine fibres, as opposed to bundles with large numbers of thick fibres. Theoretically, there is possible an enormous range of intermediate gradations and combinations—small numbers of thick fibres, large numbers of fine fibres, etc. Possibly, size of bundle as such, is less important than the coincident association of thick bands of connective tissue in coarsely grained muscle, such as has been observed by Hammond and Appleton (1932).

(*d*) Growth and Development of Muscle Fibre.

Cobb (1925), Needham (1926), Hines (1927), Denny-Brown (1929), and Hammond and Appleton (1932), have reviewed the literature dealing with the histology of muscle. Most of the original articles are not obtainable in this country. This is understandable, as Needham remarks on the fact that the field of muscle histology has been almost deserted since 1909, when attention became focussed on the chemistry of muscle.

1. *Length of fibre.*

Maximow and Bloom (1930) state that muscle fibres are entirely independent structures, of cylindrical or prismatic shape, gradually constricting towards the ends and terminating in fine points. Particularly at the union of muscle with tendon, the end of the fibre may appear rounded, notched, or provided with teeth-like projections. These authors estimate the length of striated muscle fibres may vary from 1 to 41 mm. In short muscles, the fibres may continue through the entire muscle. In the larger muscles, the fibres are usually shorter than the muscle itself, and one or both ends may lie free within the muscle.

Huber (1916-17), working with adult rabbit muscle, dissociated single fasciculi into their component fibres. He found, in muscles with relatively short fasciculi (not longer than 2·5 cm.), the fibres extend from tendon to tendon. In semi-pinnate, pinnate, or compound pinnate muscles, also where the distal and proximal tendons overlap, the respective fasciculi are much shorter than the muscle itself. No fibres longer than 2·5 cm. were seen in the longest fasciculi teased out. In other words Huber found no fibres reaching from end to end of any fasciculi longer than 2·5 cm. In longer fasciculi, the fibres had either one blunt tendon end and one filamentous intra-fascicular termination, or the fibres were spindle-shaped ending in hair-like processes within the fasciculus. It is noteworthy, in only two fasciculi of a number teased from the Gastrocnemius muscle, one single fibre was found which did not extend from tendon end to tendon end.

Lindhard (1929) measured Gastrocnemius fibres in the frog. He reports the fibre runs from one terminal tendon of the fasciculus to the other terminal tendon. It is interesting to observe differences in two species of frogs

examined. In *R. esculenta* fibres are bluntly conical, whereas in *R. temporaria* the fibres are irregularly cylindrical, arranged in pairs, a thick and a thin fibre alongside each other.

Denny-Brown (1929) says of the Gastrocnemius medialis muscle of the cat: " Careful dissection of the fresh muscle with a wet knife shows every fasciculus runs from aponeurosis to aponeurosis. It was further found . . . that in any particular fasciculus the fibres run from end to end of the fasciculus. . . . No fibre was found which did not reach from aponeurosis to aponeurosis. All fibres, thick and thin alike, found their way from end to end of the fasciculus."

Buchthal and Lindhard (1939) give an excellent review of work dealing with the anatomy of the striated muscle fibre. They establish certain general types of fibre. Thus, cylindrical or bluntly conical fibres are comparatively short. Long muscle fibres are flagelliform, or lanceolate, connected to the terminal tendons by the thick rounded end, while the tapering end is lost in the endomysium. On the average, fibres shorter than the bundle are more than half the length of the bundles. Thin fibre-ends overlap at varying points within the bundle. Varying numbers of elongated spindle-shaped fibres, with both ends terminating in the endomysium, furnish additional mechanical support.

Hammond and Appleton (1932) measured only thickness of fibre. They point out, however, the size of the muscle is determined mainly by the number or length of the fibres, rather than by their thickness.

As muscle fibres are often of considerable length it is difficult to measure their length under the microscope. Moreover, the fibres are intimately interwoven and overlapped by other fibres, so that it is almost impossible to measure their length without completely isolating the individual fibres. This process is so laborious, it is incapable of routine application. Length of fasciculus, however, is more easily determined. Where fibres pass from end to end of the fasciculus, this measurement affords an idea of the fibre length. From the evidence cited, it appears that the Gastrocnemius muscle falls within this category.

2. *Diameter of fibre.*

(i) *Technique of measurement.*

Various methods of isolating muscle fibres for measurement of the shape and the dimensions are reviewed by Buchthal and Lindhard (1939). These authors stress the difficulty in evaluating the comprehensive histological literature concerning the muscle fibre, because most observations have been made on fixed and stained fibres. Different methods have been applied for measuring muscle fibre diameter by various workers, and only in very few cases have attempts been made to examine living fibres.

Lindhard (1926) boiled the muscle *in situ* for two hours in water. After isolating individual fibres under the low-power binocular microscope, he measured uninjured fibres with the aid of an ocular micrometer.

Paff (1930) made camera lucida drawings, on squared graph paper, of transverse paraffin sections of skeletal muscles of the rat, guinea-pig, and cat. He computed the average area of muscle fibres, by counting the square millimetres enclosed by the drawn outlines of six hundred different fibres, making seventy-five measurements for each muscle.

Clark (1931) used stained celloidin cross-sections in order to count the total number of fibres in skeletal muscles of the cat. The sections were projected on bromide paper at a magnification of seventy-five to a hundred diameters, and the fibres in each photograph were counted.

Hammond and Appleton (1932) cut free-hand shavings from a formalin-fixed strip from the middle of each muscle, and teased out the shavings on a slide in a drop of dilute glycerin. Average diameter was calculated by measuring the cross-diameter of fifty fibres by means of an eye-piece micrometer. The diameters of fibres in the middle and at the end of the muscle, in ten different muscles, from four animals, averaged $40 \cdot 10\mu$ for the middles, and $42 \cdot 37\mu$ for the ends. In six out of the ten muscles the ends had the slightly thicker fibres. Hammond and Appleton conclude there may be slightly more small fibres than usual, found in measurements of cross-diameters of fibres taken from the middle of the muscle.

McMeekan (1940-41) employed essentially the same method. He stained the shavings with picric acid, and mounted them in Farrant's solution.

Robertson and Baker (1933) macerated slender strips of fresh muscle in twenty per cent. nitric acid for two to four days. The macerated muscle fibres were washed with distilled water, and mounted in glycerin. Average diameter was calculated from the measurement of two hundred fibres. In addition, fibre size was indirectly estimated, in transversely cut sections, by counting the number of muscle fibres in an area 0·207 sq. mm. Twenty-five cross-section areas of the muscle were counted in order to calculate the average number of fibres in a square.

Voss (1935) utilised two methods to measure size of fibre. In a study of the leg muscles of the frog, he used a planimeter to record the area of cross-section of the fibres at a magnification of six hundred diameters. In an extensive tabulation of muscles from the human, dog, sheep, and hedgehog, a different method was employed. Here Voss counted the number of fibres in a square millimeter of cross-section to obtain an estimate of fibre diameter.

Brady (1937) isolated fibres by micro-dissection. A filar micrometer was used to measure the diameter of fifty fibres. Satorius and Child (1938) followed Brady's technique. It is worthy of mention that fresh unfixed tissues can be examined in this way.

(ii) *Fibre diameter.*

Perusal of the literature makes it readily apparent that the causes of differences in size of muscle fibres are a matter for speculation rather than assertion.

Hammond and Appleton (1932) find that the size of dark and of clear fibres, occurring side by side in the same muscle, varies independently of the colour of the fibres. Average size of fibre varies from muscle to muscle, but there is a more or less constant difference between the relative size of fibres in the different muscles. Denny-Brown (1929) maintains that there is no histological criterion of the speed of contraction of a muscle fibre. Although redness is generally associated with slowness of contraction, this is only a chance association with many exceptions. There is no relation between histological features, such as thickness or thinness of individual fibres, redness or paleness, and the rapidity or slowness of contraction. Voss (1935)

tested the correlation between fibre size and delicacy of movement of the muscle. He believes the more delicate the motion of which a muscle is capable, the finer is the degree of sub-division of the contractile mass.

Hammond and Appleton (1932) are of opinion that the differences in growth and development of muscles are determined by the interplay between complex factors such as evolution, function, and rate and degree of post-natal growth. Muscles used mainly for movement have on the whole smaller fibres than those used for maintaining posture probably because the small size of the fibre facilitates quick respiratory exchange (small pale fibre). Generally speaking, in the evolution of a muscle increasing in the history of a species, there is an increase in the number of fibres rather than their size, in order to increase activity and function of the muscle. With regard to rate and degree of post-natal growth, the earlier differentiating muscles tend to increase in size of fibre alone, whereas the latter differentiating muscles tend to be developed in number of fibres as well as size of fibre. On the other hand, in the individual after birth increase in number of fibres is not possible. Consequently, with increase of muscle function, an hypertrophy of the fibres occurs, together with an extra supply of myoglobin to facilitate respiratory exchange (large red fibre).

Donaldson (1915) cites Morpurgo's (1898) data regarding the number of muscle fibres in M. Radialis of the albino rat, from which it would appear that the fibres have increased by twenty-three per cent. at fifteen days, as compared with the new-born animal. Thereafter, until 420 days of age, cell multiplication is insignificant. Schultz (1934) reports that the muscle fibres of the frog increase in number with increasing age. She finds this post-natal increase proceeds more rapidly in younger than in older frogs, and continues until the number of muscle fibres is doubled. However, Hammond and Appleton (1932) state muscle growth after birth is mainly due to increase in size of the muscle cells although they were unable to determine precisely at which stage muscle cell formation ceases in the sheep. McMeekan (1940-41) is unable to detect any increase in the number of fibres per bundle, in pig muscles, from birth to twenty-four weeks of age. Eliot, Wiggington and Corbin (1943) find that the number of muscle fibres in M. Soleus of the rat is not influenced by age of the animals. Concensus of opinion seems to favour this point of view, that growth of muscle occurs by hyperplasia in pre-natal life, and by hypertrophy in post-natal life (MacCallum, 1898; Schiefferdecker, 1919). Hence, it is to be expected that fibre diameter increases as the animal becomes older.

Apart from this thickening with age, good nutrition also increases the size of the muscle fibre. Conversely, defective nutrition reduces fibre diameter. Thus, Robertson and Baker (1933) find that muscle fibres of full-fed yearling steers are greatest in diameter and rough-fed smallest, while fibres from half-fed steers are intermediate in size. Black *et al* (1931) show that muscle fibres from steers fed a supplementary ration are slightly larger than those from steers on grass alone. Primitive breeds of sheep kept under poor nutritive conditions—semi-wild Shetland 45·5μ—have smaller muscle fibres than a highly improved breed reared on high nutrition—Suffolk 49·2μ (Hammond and Appleton, 1932). Similarly, McMeekan (1940-41) observes that pigs reared on a high plane of nutrition until sixteen weeks, have fibres roughly fifty per cent larger than individuals of the same breed reared on low nutritive conditions (12·08μ–8·52μ). Moreover, this difference in fibre diameter is closely related to differences in the weights of both pig and muscle.

Kremer (1930) indicates that the musculature acts as a food reservoir in the hibernating frog. In consequence, the striated muscle is altered as this reserve is used. Voss (1937) maintains that starvation decreases fibre thickness in the muscles of the frog. Greene (1912), in an extremely interesting study, observes that the king salmon stores large quantities of fat in the muscular tissues, during its life in the ocean. It ceases to take food when it enters the fresh waters of the rivers in the journey to the spawning ground. Fat is gradually removed from the muscle during the migration period, so that it has almost disappeared when the fish has reached the spawning stage. Verne (1938) reports a marked decrease in the lipids in muscle fibre during fasting. Bell (1909) and Bullard (1916) describe lipoidal granules in muscle fibres, which are increased by feeding and reduced by starvation. Denny-Brown (1929) shows an increased granulation in the muscle of fattened cats, whereas the granulation seems to vanish in emaciated muscle.

As regards sex differences in size of muscle fibre, Eliot, Wiggington and Corbin (1943) observed no difference in size of fibre in M. Soleus of male and female rats. However, Hammond and Appleton (1932) report that the ram has larger fibres than the ewe, and wethers have fibres intermediate in size. Mehner (1938) states the muscle fibres, from M. Gracilis and M. Sartorius of chickens, are larger in the male. On the other hand, Brady (1937) and Satorius and Child (1938) find that cows have significantly larger fibres than steers. It must be remarked that their experimental material comprised six Hereford-Shorthorn yearling steers and seven mature Holstein cows. As both age and breed are known to influence fibre diameter, it is unfair to attribute this difference to sex alone.

The effect of breed differences have been studied by Hammond and Appleton (1932). These authors show that the muscle fibres are larger in an improved breed of sheep than those of an unimproved breed. They believe there has also been an increase in the number of fibres in each muscle in the improved breed. They cite Malsburg (1911) to the effect that the heavier breeds of farm animals have larger fibres than the lighter breeds. Mehner (1938) confirms this finding. In a study of twelve races and crosses of ninety chickens, he finds the distinct racial differences in diameter of muscle fibre are almost parallel to the racial differences in body size. Mehner is of opinion the variations in size of the muscle fibres are almost enough by themselves to account for the differences in body size.

The comprehensive literature dealing with the effect of exercise on muscle has been reviewed by Steinhaus (1933). Various aspects of the problem have been investigated by Eliot, Wiggington and Corbin (1943), Fischer (1940), Bruman and Jenny (1936), Petrén (1936), Petrén *et al* (1936), Frey (1936), Rein et al (1935), Donaldson et al (1932, 1933), Donaldson [1935 (a), 1935(b)], Vannotti and Mageday (1934), Thörner (1930, 1934) Vannotti and Pfister (1933), and Regnault (1927). Consensus of opinion indicates that increased exercise produces increased vascularisation and hypertrophy of muscle. Steinhaus cites Siebert (1928), who states exercises of speed, strength, effort, induce hypertrophy of skeletal muscle, whereas exercises of endurance leave the body muscles unchanged in size. Morpurgo (1897), attributes hypertrophy to true enlargement of existing fibres solely due to formation of an increased amount of sarcoplasm. Thus, there is "no change in fibre length nor in the number of nuclei, nor the number or size of the fibrilli in the muscle cell."

(iii) *Colour or structure of muscle fibre.*

Colour of meat in relation to breed, condition, age, sex, feeding, management, exercise, and storage, is discussed by Hirzel (1939). In the higher mammals all muscles with few exceptions are red, but differences exist in the degree of redness between different muscles, also under different environmental conditions. For example, it has been found that the thigh muscles of the sheep are paler than the leg muscles. Moreover, the redness of these muscles increases with age and activity (Hammond and Appleton, 1932; Griffiths, Vickery, and Holmes, 1932). This redness is due to the haemoglobin content (myoglobin) of the muscle (Kühne, 1865; Whipple, 1926).

Millikan (1939) states: "Muscle haemoglobin is generally found in large quantities in those muscles requiring slow repetitive activity of considerable force." Hammond (1942) deduces the fatigue-resisting function of myoglobin, from the dark red colour of muscles of game animals living an active life, such as the hare, grouse, deer. These animals have darker coloured muscles than the domesticated rabbit, fowl and sheep. Within the same animal, colour differences may be explained on a like basis. For example, in the leg of the sheep, the muscle Extensor pedis which functions continually in maintaining posture is dark red in colour (Hammond and Appleton, 1932 (p. 497). In the rabbit, Roberts (1916) believes the red muscles play a prominent part in maintaining posture and fixing joints (M. Soleus, M. Crureus, deep head of M. Triceps).

Mention has been made of the extensive use of frog muscle for physiological investigation of muscle contractility. Although this muscle is pale and unpigmented, histological study reveals the presence of granular (sarcoplasmic, protoplasm-rich) and clear fibres (aplasmic, protoplasm-poor). Earlier workers attempted to homologise these two types of muscle fibre with the red and white muscles of birds and mammals.

Early workers studied especially the red and white muscles in the rabbit. They were inclined to homologise these two types with the histologically different dark and clear muscle fibres. Morphological differentiation was based chiefly on the relatively greater amount of sarcoplasm in the dark fibre, also the fact that the nuclei are not always found immediately beneath the sarcolemma as in the clear fibre. Hines (1927) cites Schaffer (1893), who reported also that the clear fibres in man contain small myofibrils arranged rather regularly, whereas in the granular fibres the fibrils are large and the arrangement without order.

Although striated muscle of higher vertebrates is red in colour, both dark and clear fibres are present so that few muscles are exclusively "red" or "white" in their make-up. Hammond and Appleton (1932), in a macroscopic examination of the leg muscles of the sheep, find all shades of colour between red and white linking up the extremes. Microscopical examination of these muscles showed that the proportion of dark, clear, and intermediate fibres varies according to the colour of the muscle, but intermediate fibres are numerous in practically all muscles.

Contradictory evidence is presented regarding age changes in the colour of muscle fibres. Denny-Brown (1929) states that the fibres of the pale muscle of the new-born kitten appear dark in cross-section due to the presence of numerous granules of some complex lipoidal substance. At fourteen days a proportion of fibres are clear and by a continuation of the process the

muscle ultimately becomes a mixture of dark and clear fibres. On the other hand, Hammond and Appleton (1932) find only clear fibres in the muscles of the newly born lamb. Later, in the five month old sheep, the majority of fibres are clear and intermediate in colour. In the adult sheep at twenty-two months, the full colour of the dark fibres has developed. The muscle fibres are on the whole darker than those of the younger animal, with a corresponding increasedly marked contrast in colour of dark and clear fibres. It is to be noted, in spite of the pale colour of the muscles in the lamb at birth, the fibres are structurally dark, with scattered nuclei and much sarcoplasm. McMeekan (1940-41) notes the presence of fat globules within certain muscle fibres of the newly born pig. These globules gradually decrease with age and cannot be detected after sixteen weeks. No specific mention is made, but presumably little alteration in colour of muscle fibres occurs up to twenty-eight weeks of age, as the muscle itself remains almost colourless throughout.

Owing to the confused state of knowledge, Cobb (1925) emphasises the need for more work before accepting physiological interpretations concerning red and white muscle. He suggests redness is not an essential feature. For example, colour varies in the same species (rabbit, hare) and genus (sedentary person, athlete). Cobb is of opinion histological criteria should rather be employed for differentiation of muscles i.e. primitive small fibres with centrally placed nuclei, as opposed to the more highly developed type with larger fibres, less sarcoplasm, and peripherally placed nuclei.

Lebedeva (1930) disregards colour differences. He considers muscles in the rabbit and cat fall into two classes. Dynamic muscles consisting of long parallel fibres have the typical structure of " white " muscle. Static muscles consisting of short fibres exhibit mostly a typical structure of " red " muscle.

Hammond and Appleton (1932) too, are inclined to the view that colour is not necessarily related to the structure of the fibre. They suspect " the differences in structure described in muscle fibres are the result of the degree of specialisation during development . . . differentiation rather than increase in size ".

Hammond and Appleton cite Lewis and Stohr (1913) to the effect that enlargement of fully formed fibres takes place by increase of sarcoplasm. Similarly, Steinhaus (1933) quotes evidence to indicate hypertrophy is attributable to an increased amount of sarcoplasm. It follows therefore, that the histological appearance will be altered as a result of muscle hypertrophy, owing to the formation of an increased amount of sarcoplasm between the fibrilli of the fibre. Quite apart from this quantitative improvement of trained muscle, it may be expected that there will be a qualitative improvement by the formation of an increased amount of myoglobin in the hypertrophied muscle, with consequent reddening of the muscle. In fact, Bloor and Snider [1934(a), 1934(b)] show that additional muscular function results not only in a large increase of myoglobin, but in an increased phospholipid content as well. It is clear additional experimental evidence is required, of the effect on the micro-structure of muscle fibre of hypertrophied muscle, of these so closely associated responses to activity.

CHAPTER III.—PLAN OF INVESTIGATION.

(*a*) Animals.

Albino rabbits were purchased from four different local breeders prior to February, 1940 [July-August, 1938, 51; November, 1938, 46: March, 1939, 30; January, 1940, 19].

A small number of the outstanding animals from each batch was selected for breeding purposes by the officer in charge of the small animal establishment. Of the remainder, the majority were sacrificed for experimental requirements at this Institute.

In-breeding of the progeny of the original breeding stock was applied after January, 1940, to satisfy the local demand for experimental rabbits. All the animals used for this investigation were derived from the original stock, the earliest used in the experiment being bred in August, 1940, the last in November, 1943.

(*b*) Housing, management, and feeding.

Throughout the period of these investigations, the housing and management of the rabbits were unchanged.

From the time of weaning, each rabbit lived alone in a separate outdoor run communicating with a concrete hutch. The accompanying photos (Plates I and II) adequately describe the accommodation. However, dimensions are stated for the sake of completeness. A hinged iron roof covers the concrete hutch, which is 2 feet 6 inches long, 16 inches wide and 2 feet 6 inches high. Openings 6 inches wide, across the top of the front and back walls facilitate ventilation, and an opening at the bottom of the front wall allows entry into the run. This run, 6 feet long and 20 inches wide, also made of concrete, is enclosed by wire mesh. Attendants obtain access to the run by lifting the hinged wire mesh cover of the run. For drinking purposes, water was always available in a shallow trough outside in the run, whereas inside the hutch, a plentiful supply of clean veld hay was maintained for bedding. Throughout the year the small animal establishment is sheltered by the surrounding hedges. In summer particularly, shade is provided by poplar trees planted along the rows of rabbit houses. During the period of this experiment the rabbits were free of sickness. Except at parturition the mortality was nil, adequate testimony to their living conditions.

Freshly cut green foodstuff—lucerne or barley—was given at 8 to 8.30 a.m. each morning. In general, lucerne was supplied during the winter months, and barley in summer, but it largely depended upon what crop was available at the time. A liberal supply of the dry mash usually fed to rabbits at this Institute was placed in the food-pan at 2.30 p.m. daily, except in rainy weather when no dry ration was supplied. At 6.30 a.m. the following morning the remains were discarded. At this time the run was cleaned out, and the food-pan was washed and left outside the run until next feeding time.

This mash was composed as follows:—

Oats	65 per cent.
Wheat bran	20 per cent.
Linseed meal	12 per cent.
Bonemeal	2 per cent.
Lime	0·5 per cent.
Salt	0·5 per cent.

Because feeding of bran to animals was prohibited after June 1941 as a war measure, the mash comprised the following materials from that date:

Yellow maize meal—5 parts	60 per cent.
Crushed maize—1 part	
Crushed oats—2 parts	12 per cent.
Rolled oats—1 part	
Linseed meal	10 per cent.
Lucerne meal	10 per cent.
Meat meal	5 per cent.
Bonemeal	2 per cent.
Salt	0·5 per cent.
Lime	0·5 per cent.

Breeding does and bucks were housed essentially as described, except that the hutch and run had twice the floor space. When the doe was on heat, she was taken to the buck's hutch and removed after two " falls ". Records were kept of the date young rabbits were born, their sex, the number in the litter, and the nest was inspected daily for some while after parturition.

Weaning of the young rabbits was carried out at seven to eight weeks. Sex was determined at this stage, and the buck rabbits were removed to their new homes, after weighing them. Identity of each rabbit was secured by tattooing a serial number in the right ear, by means of tattooing forceps.

For a variety of reasons, the young rabbits were at first weighed only after weaning. Later it became the practice to start weighing one day after birth, and to make daily weighings until seven weeks of age. Subsequent to this, weighings were carried out at weekly intervals until the rabbits were killed for the purpose of this experiment.

Weighing was always performed at the same time of the day, before the rabbits received their green food at 8 to 8.30 a.m., and the same day of the week—Saturday—was utilised for the weekly weighing. Circumstances with regard to food and time of day were thus as nearly uniform as possible.

It was rarely necessary to abandon weighing on account of rain, but on such occasions the weekly weighing was carried out on Sunday or Monday. If this was not possible, weighing was postponed until the following Saturday. In such cases, the average growth was obtained by dividing the total amount gained by two.

(*c*) Nature and scope of the experiment.

A complete study of post-natal growth and development of muscle should embrace observations from birth to death. Considerable time would elapse before the completion of such work, as senile material would only be available maybe six years after the birth of animals destined for that purpose. Furthermore, the limited facilities available for rearing rabbits (twenty hutches only) would severely limit the number of experimental animals.

Preliminary observations established the maximum weight of the strain of albino rabbits used as more or less 3,000 gm. Animals could be regarded as adult when they attained this weight. It was decided to investigate the

muscle growth accruing as a result of an arbitrary live-weight increase of 600 gm., commencing with the newly-born rabbit. Thus, observations were made on muscle taken from newly-born rabbits, at 600 gm. live-weight, and at 1,200, 1,800, 2,400 and 3,000 gm. live-weight. In addition, rabbits were kept for a period of six months after they first attained 3,000 gm. body weight, in order to determine changes as the animals matured.

When the data from these animals were analysed, it became evident that additional observations were desirable between birth and 600 gm. live-weight, in respect of certain muscle characters. Accordingly five additional groups were taken at 100 gm. live-weight, 150 gm., 220 gm., 320 gm., and 480 gm.

In growth studies, the most desirable data consist of measurements taken at comparable stages on a series of individuals followed throughout their period of development. This is obviously impossible in a study of this nature, where the animals must be killed in order to obtain the data. It must be assumed the older rabbits are what those killed at the earlier ages would have become at a later stage of their lives.

The chances of overcoming the inherent variability of the experimental material improve as the number of animals is increased. However, by means of inbreeding, it becomes possible to draw valid conclusions from smaller numbers of animals, as great uniformity is achieved. It was decided to utilise ten individuals for each weight-class. All observations recorded are, therefore, the means for ten rabbits within each group, with the exception of the groups from 100 gm. to 480 gm. live-weight, included later in the experiment. Here only five individuals were utilised for each group, as the data already collected seemed to justify this step.

In order to eliminate sex variation as a source of error, only buck rabbits were employed for this study. It is hoped it will be possible to compare muscle growth in does at some future date, to determine the effect of sex.

Environmental variations would have been minimised if all required experimental animals had been bred at the same time and reared together, until random selection of individuals for the different weight-groups. However, the facilities available rendered it impossible to breed and rear in this manner the large number of rabbits required. Consequently breeding was carried out the whole year round. Individuals were allocated at random whenever necessary. Each experimental group therefore comprised animals born at different seasons, and during different years for different groups.

It was estimated it would not be possible to study more than two muscles by the methods proposed. The problem was which individual muscles should be selected for investigation. Both muscles should be capable of easy separation to permit of accurate measurement, yet, if possible, the muscles should have well-defined individual action. M. Psoas major was selected as a late developing muscle from the region of the back and loin. For contrast, M. Gastrocnemius medialis in the leg was chosen as the second muscle. Both muscles are easily identified and removed, moreover they represent different types of muscle.

M. Gastrocnemius medialis is a reddish muscle in which the muscle substance lies between two fascial layers; " one, an aponeurosis attached at its upper end to the femur and fading into muscle substance at its lower end; the other, an aponeurosis running into the Tendo Achilles below and

PLATE I.

A photograph taken in 1939, showing a general view of the small animal section. Similar accommodation is provided for rabbits.

PLATE II.

Roof of hutch and wire mesh cover of run in the raised position.

fading into the muscle substance above." (Denny-Brown, 1929). Hundreds of fasciculi pass from the aponeurosis of origin (which is superficial), obliquely downwards and forwards to the aponeurosis of insertion. Hence fasciculus length is much shorter than muscle length in this case.

On the other hand, the pale Psoas major muscle is much larger than M. Gastrocnemius medialis. It is characterised by the largely parallel arrangement of its fibres along the long axis of the muscle, from origin to insertion. Origin is by means of a broad aponeurosis from the pleural surface of the third last rib, near the costal angle; also from the body of the last thoracic and all lumbar vertebrae, as well as from the transverse processes of the last-named. The muscle passes caudally along the long axis of the body, to unite with the terminal portion of M. Iliacus to form a common stout tendon inserted on the trochanter minor of the femur (Gerhardt, 1909).

Both M. Psoas major and M. Gastrocnemius medialis were studied as minutely as possible, in terms of the morphology of each functional unit. Firstly, the individual muscle was studied as a whole, in terms of weight, length, width, and depth; secondly, the individual bundle in terms of length and thickness; and lastly, the individual fibre, also as regards length and thickness.

(*d*) Procedure.

1. *Collection of data.*

Rabbits were killed as soon as possible after they had attained the required weight. As Saturday was the regular day for weighing rabbits, it usually happened that the animals were killed on Monday morning. They were removed from the hutches before feeding, with as little disturbance as possible, to the laboratory a short distance away.

In order to minimise effects due to struggling, it was customary to anaesthetise the rabbit before bleeding it. Nembutal solution, utilised for this purpose, was injected intravenously at the rate of 0·2 c.c. per pound body weight. In addition, ether was lightly administered by means of a mask to deepen the degree of anaesthesia attained.

When relaxation was complete the carotid arteries and jugular veins were severed and the rabbit was suspended by the tail for fifteen to thirty minutes for drainage of the blood. At this stage the position was inverted, and the rabbit was suspended from both fore-limbs for approximttely five hours. This measure was undertaken to ensure the hind legs and body were in a position as nearly reproducible as possible, during the period of rigor mortis allowed.

Selection of the period of rigor for five hours was arbitrary. It was designed to ensure loss of muscle irritability before handling, as well as to permit dissection and weighing of the muscles during the routine working hours. Data presented by Bate-Smith (1939) indicate it would be desirable to allow a longer period of rigor mortis. His work on the Psoas muscles from rabbits indicates that stiffening occurs up to eight hours after death, inclusive of an initial " normal " stationary period of two hours. Unfortunately it was not possible to utilise an eight-hour period. Leaving the animal overnight at room temperature did not offer a solution, as the degree of autolysis attained made it difficult to perform the required measurements on isolated muscle bundles. Experiments were also undertaken to achieve a reproducible standardised condition of the muscles, by perfusing the

anaesthetised animal with formalin solution. As living muscle reacts strongly to perfused fixative, this method too is not without disadvantage, and the method described was preferred.

After rigor mortis had progressed for five hours, the right Gastrocnemius medialis muscle was removed, weighed immediately, and immersed in neutralised ten per cent. Formalin solution in normal saline. Then followed in turn, the left Gastrocnemius medialis and right and left Psoas major muscles. Each muscle was removed from the fixative after 24 to 48 hours, rinsed in water, and the excess water mopped off the muscle. After measuring length of muscle to the nearest millimetre, the muscle was marked off into six equidistant portions demarcated as follows:—

Origin to " A ", " A " to " B ", " B " to " C "; " C " to " D ", " D " to " E ", and " E " to insertion. In Figure 1, the procedure is explained in diagrammatic manner. Width of muscle was measured by means of a vernier caliper at points, A, B, C, D, E, after laying the muscle flat on top of the bench. Depth of muscle was measured by means of the same instrument, also at these five points, but the muscle was lifted for this measurement.

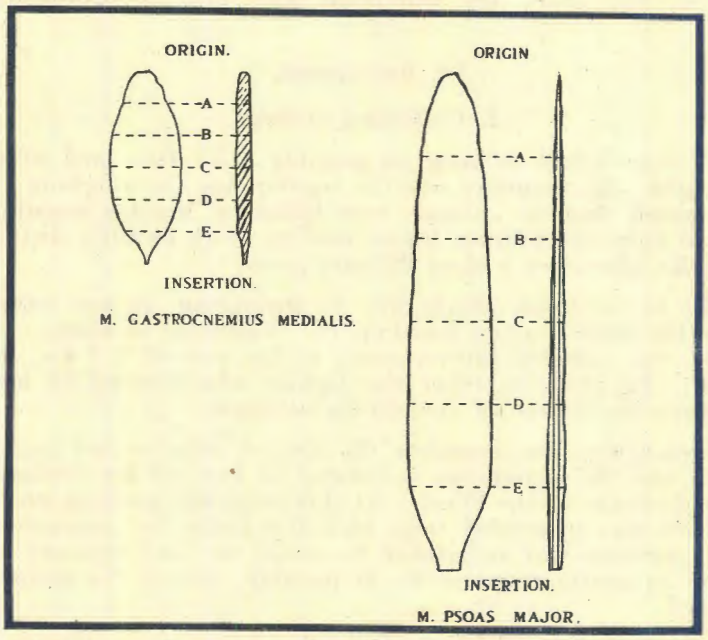

Fig. 1.—Sites at which width and depth of muscle were measured. The shading indicates the direction of muscle bundles on sectioning the muscle longitudinally.

Length of muscle bundle was always measured on the left muscle. In the case of M. Gastrocnemius, bundles were carefully teased out under water, at sites A to E, from a central longitudinal strip of muscle. Statistical methods were employed to ascertain suitability of sampling. These data, as well as the method of measurement, are presented later (page 355). The Psoas muscle could not be treated similarly because of the end-to-end arrangement of its component bundles. In this muscle it was only necessary to measure bundles along the length of the muscle.

Fibre diameter was determined on specimens removed from the right muscle, at sites A to E. A statistical evaluation of the suitability of method employed to measure fibre diameter is considered in detail at a later stage (page 360).

In addition, specimens were removed from the right muscle for histological purposes. These specimens were cut out immediately cranial to site C to ensure that each specimen was obtained at a relatively constant position and furthermore from the centre of the muscle where material is not scanty. " Grain ", or texture of muscle was estimated from these pieces of muscle, by the method later described (pages 365 and 399). The specimens were washed in running water for several hours to remove excess Formalin solution. After infiltration with ten per cent. gelatin solution at 37° C. for roughly sixteen hours, the specimens were transferred to twenty per cent. gelatin for twenty-four hours, then imbedded in gelatin of like concentration. Setting of the gelatin was achieved by holding in a refrigerated chamber at roughly 1–5° C. for two to three hours. Excess gelatin was trimmed from the specimen, which was then transferred to cold ten per cent. neutral solution of Formol-saline to harden. Frozen sections were cut at 35μ by means of a Jung microtome equipped with a carbon dioxide attachment.

Measurement of length of muscle bundle.

Preliminary work showed how laborious it would be, as a routine measure, to dissect out a large number of fasciculi in order to determine the mean length of the muscle bundles. It became necessary therefore, to determine the minimum number of measurements likely to afford a reasonably accurate estimate of bundle length.

In the case of the Gastrocnemius muscle, specimens were always obtained from a two millimetre wide central longitudinal strip of the muscle, in order to ensure a relatively constant site. After marking the superficial muscle surface at equidistant points to give three equal portions, the fasciculi in each third were carefully dissected out under water. Immediately after their removal, the bundles were straightened on the table top and calipers were used to measure their length to the nearest millimetre. Fifty random fasciculi were measured in each third of the muscle, and the data analysed.

Because of the end-to-end arrangement of bundles, the Psoas muscle could not be treated similarly. In this muscle a central strip was again used. The bundles pass right along the length of this strip, from which seventy bundles were removed at random for measurement.

These numbers are too large to be practical in experiments involving a number of animals. Hence the minimum number of measurements that can be used to give a mean value, that is representative of the muscle, was determined.

An analysis of the Gastrocnemius measurements is given in Tables 1 (A) and 1 (B), where the selection of fifty bundles at each site within the muscle is compared with selections of forty, thirty and twenty measurements. In addition, five differing selections each consisting of ten bundles are shown with their means and other statistical constants, as well as the results of the tests for significance. The Psoas measurements are presented in Tables 2 (A) and 2 (B), where seventy measurements are compared with selections of sixty, fifty, forty, thirty, and twenty, in addition to seven differing selections each consisting of ten measurements.

TABLE 1A.

Analysis of measurements of length of muscle bundles. Gastrocnemius muscle. No. 1.

Length of Bundle (mm).	Number of Bundles in Each Selection.									
	50.	40.	30.	20.	20.	10.	10.	10.	10.	10.
PROXIMAL THIRD.										
10	2	1	1	1	1	—	1	—	—	1
11	37	29	21	16	14	8	8	8	6	7
12	11	10	8	3	5	2	1	2	4	2
Mean (mm)	11·2	11·2	11·2	11·1	11·2	11·2	11·0	11·2	11·4	11·1
Standard error	±·0681	±·0759	±·0920	±·1000	±·1170	±·1332	±·1491	±·1334	±·1633	±·1795
Standard deviation	·4818	·4798	·5041	·4472	·5232	·4217	·4714	·4217	·5164	·5676
Coefficient of variability	4·3%	4·3%	4·5%	4·0%	4·7%	3·8%	4·3%	3·8%	4·5%	5·1%
Significant differences	—	—	—	—	—	—	—	—	—	—
MIDDLE THIRD.										
10	1	1	1	1	—	—	—	—	1	—
11	24	19	16	10	9	3	5	6	4	6
12	25	20	13	9	11	7	5	4	5	4
Mean (mm)	11·5	11·5	11·4	11·4	11·5	11·7	11·5	11·4	11·4	11·4
Standard error	±·0769	±·0876	±·0891	±·1338	±·1141	±·1527	±·1667	±·1663	±·2211	±·1633
Standard deviation	·5436	·5543	·5632	·5982	·5104	·4830	·5271	·5164	·6992	·5164
Coefficient of variability	4·7%	4·8%	4·9%	5·3%	4·4%	4·1%	4·6%	4·5%	6·1%	4·5%
Significant differences	—	—	—	—	—	—	—	—	—	—
DISTAL THIRD.										
11	1	1	1	—	1	—	—	—	—	1
12	27	22	18	13	9	7	4	7	5	4
13	19	15	8	5	9	3	5	2	4	5
14	3	2	3	2	1	—	1	1	1	—
Mean (mm)	12·5	12·4	12·4	12·4	12·5	12·3	12·7	12·4	12·6	12·4
Standard error	±·0914	±·1010	±·1151	±·1535	±·1539	±·1527	±·2134	±·2211	±·2211	±·2211
Standard deviation	·6465	·6385	·7280	·6863	·6882	·4830	·6749	·6992	·6992	·6992
Coefficient of variability	5·2%	5·2%	5·9%	5·5%	5·5%	3·9%	5·3%	5·6%	5·6%	5·6%
Significant differences	—	—	—	—	—	—	—	—	—	—

TABLE 1B.

Analysis of measurements of length of muscle bundle. Gastrocnemius muscle. No. 2.

Length of Bundle (mm).	Number of Bundles in Each Selection.									
	50.	40.	30.	20.	20.	10.	10.	10.	10.	10.
PROXIMAL THIRD.										
11	11	10	8	4	4	—	1	1	4	5
12	28	22	17	10	12	7	9	6	4	2
13	11	8	5	6	4	3	—	3	2	3
Mean (mm)	12·0	12·0	11·9	12·1	12·0	12·3	11·9	12·2	11·8	11·8
Standard error	±·0947	±·1071	±·1208	±·1606	±·1451	±·1527	±·1000	±·2000	±·2494	±·2906
Standard deviation	·6698	·6774	·6617	·7181	·6488	·4830	·3162	·6324	·7888	·9189
Coefficient of variability	5·6%	5·7%	5·6%	5·9%	5·4%	3·9%	2·7%	5·2%	6·7%	7·8%
Significant differences	—	—	—	—	—	—	—	—	—	—
MIDDLE THIRD.										
11	5	4	2	3	2	1	1	3	—	—
12	28	24	16	12	10	4	6	5	7	6
13	17	12	12	5	8	5	3	2	3	4
Mean (mm)	12·2	12·2	12·3	12·1	12·3	12·4	12·2	11·9	12·3	12·4
Standard error	±·0885	±·0961	±·1109	±·1433	±·1469	±·2211	±·2000	±·2333	±·1527	±·1633
Standard deviation	·6259	·6076	·6073	·6407	·6568	·6991	·6324	·7379	·4830	·5163
Coefficient of variability	5·1%	5·0%	4·9%	5·3%	5·3%	5·6%	5·2%	6·2%	3·9%	4·2%
Significant differences	—	—	—	—	—	—	—	—	—	—
DISTAL THIRD.										
12	3	1	1	2	1	—	1	—	1	1
13	38	32	23	16	14	6	7	9	9	7
14	9	7	6	2	5	4	2	1	—	2
Mean (mm)	13·1	13·2	13·2	13·0	13·2	13·4	13·1	13·1	12·9	13·1
Standard error	±·0679	±·0675	±·0844	±·1026	±·1170	±·1633	±·1795	±·1000	±·1000	±·1795
Standard deviation	·4802	·4266	·4621	·4588	·5231	·5163	·5676	·3162	·3162	·5676
Coefficient of variability	3·7%	3·2%	3·5%	3·5%	4·0%	3·9%	4·3%	2·4%	2·5%	4·3%
Significant differences	—	—	—	—	—	—	—	—	—	—

TABLE 2A.

Analysis of measurements of length of muscle bundle. Psoas muscle. No. 1.

Length of Bundle. (mm).	Number of Bundles in Each Selection.															
	70.	60.	50.	40.	30.	30.	20.	20.	20.	10.	10.	10.	10.	10.	10.	10.
84.....	2	2	1	1	1	—	—	1	—	—	1	—	—	—	—	1
85.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
86.....	2	2	1	1	2	—	2	—	—	—	—	—	1	1	—	—
87.....	2	2	2	1	1	1	1	1	—	—	—	—	1	—	1	—
88.....	3	3	2	1	—	2	2	—	1	1	1	—	—	—	1	—
89.....	2	1	2	1	1	1	1	—	—	1	—	1	—	—	—	—
90.....	1	1	1	—	1	—	1	—	—	—	—	—	—	1	—	—
91.....	2	2	2	2	1	1	2	—	—	—	—	1	—	—	—	1
92.....	3	1	1	1	1	2	2	1	—	1	—	—	—	—	1	1
93.....	3	3	1	2	2	—	—	1	1	—	—	—	1	1	1	—
94.....	5	5	2	2	2	2	—	2	2	2	—	1	—	—	1	1
95.....	8	8	5	6	3	4	1	4	3	3	—	2	1	1	1	—
96.....	5	2	4	3	2	3	—	4	1	—	1	—	2	1	—	1
97.....	11	9	9	7	4	5	3	1	7	1	1	4	—	2	1	2
98.....	7	5	6	6	3	3	1	2	2	1	2	1	1	2	—	—
99.....	2	2	1	—	1	1	—	1	1	—	—	—	—	—	1	1
100....	6	6	5	3	2	3	2	1	1	—	2	—	2	—	1	1
101....	4	4	3	1	1	2	2	1	1	—	1	—	1	1	1	—
102....	2	2	2	2	2	—	—	—	—	—	1	—	—	—	—	1
Mean...	95·03	95·02	95·38	95·30	94·73	95·43	93·30	95·05	96·25	93·70	96·40	95·00	95·20	95·10	94·60	95·20
S. E...	±·5361	±·6072	±·6407	±·6662	±·8879	±·7123	±1·1700	±·8929	±·6236	±1·0116	±1·8511	±·9309	±1·6519	±1·3861	±1·5144	±1·6519
S. D...	4·4850	4·7030	4·5306	4·2135	4·8632	3·9012	5·2324	3·9931	2·7886	3·1989	5·8538	2·9439	5·2238	4·3831	4·7889	5·2238
C. V...	4·7%	5·0%	4·8%	4·4%	5·1%	4·1%	5·6%	4·2%	2·9%	3·4%	6·1%	3·1%	5·5%	4·6%	5·1%	5·5%
Sig. Diff.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations :—

S.E..... Standard error.
S.D..... Standard deviation.
C.V..... Coefficient of variability.
Sig. Diff..... Significant difference.

Table 2B.

Analysis of measurements of lenght of muscle bundle. Psoas muscle. No. T.

Length of Bundle. (mm).	Number of Bundles in Each Selection.															
	70.	60.	50.	40.	30.	30.	20.	20.	20.	10.	10.	10.	10.	10.	10.	10.
90.....	2	2	—	2	1	—	—	—	—	—	—	—	1	—	—	1
91.....	10	10	9	3	5	4	6	2	2	1	2	1	2	1	2	1
92.....	7	6	6	4	2	4	3	1	1	3	—	1	—	1	—	2
93.....	11	8	10	9	4	5	1	6	3	1	1	2	1	4	2	—
94.....	13	12	8	7	6	5	5	1	5	2	1	3	—	1	2	4
95.....	7	6	5	5	2	4	1	4	1	1	3	1	—	1	—	1
96.....	5	4	3	1	2	3	1	3	—	1	1	—	1	—	2	—
97.....	7	5	3	5	3	3	2	1	4	1	—	1	1	2	1	1
98.....	3	3	3	1	3	—	1	1	1	—	—	1	1	—	1	—
99.....	3	2	1	3	1	1	—	—	2	—	2	—	1	—	—	—
100.....	1	1	1	—	1	—	—	—	1	—	—	—	1	—	—	—
101.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
102.....	1	1	1	—	—	1	—	1	—	—	—	—	1	—	—	—
Mean.....	94·23	94·12	94·04	94·18	94·37	94·23	93·40	94·55	95·10	93·60	94·80	94·10	95·70	93·80	94·30	93·30
S. E.....	±·3121	±·3437	±·3681	±·3821	±·4971	±·4641	±·5099	±·5780	±·6065	±·6182	±·8794	±·6741	±1·3337	±·6289	±·7608	±·6506
S. D.....	2·6108	2·6622	2·6027	2·4166	2·7226	2·5419	2·2804	2·5850	2·7125	1·9550	2·7808	2·1316	4·2176	1·9888	2 4060	2·0575
C. V.....	2·8%	2·8%	2·8%	2·6%	2·9%	2·7%	2·4%	2·7%	2·9%	2·1%	2·9%	2·3%	4·4%	2·1%	2·6%	2·2%
Sig. diff.....	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations :—

S.E..... Standard error.
S.D..... Standard deviation.
C.V..... Coefficient variability.
Sig. Diff..... Significant difference.

No significant differences are shown among the means obtained from these various selections of measurements.; Furthermore, for each selection of ten measurements, the probable range of the mean does not exceed three per cent., which falls within the five per cent. level regarded as a satisfactory biological standard (Table 3). It was concluded that a reasonably accurate measurement of length of muscle bundle is obtained by measuring ten bundles. Accordingly all calculations in the growth study were based on an average value obtained from ten bundles.

TABLE 3.

Probable range of mean bundle length. Analysis of variance calculated at the 5 per cent. level of probability for five (or seven) samples of ten measurements each, in two muscles.

Muscle.	Number of Measurements.	Mean Bundle Length (mm.).	Probable Range of Means of 10 Measurements.
			Per Cent.
Gastrocnemius No. 1.—			
Proximal third	50	11·18	2·30
Middle third	50	11·48	2·57
Distal third	50	12·48	2·80
Gastrocnemius No. 2.—			
Proximal third	50	12·00	2·94
Middle third	50	12·24	2·70
Distal third	50	13·12	1·90
Psoas No. 1	70	95·03	2·57
Psoas No. 2	70	94·28	1·46

In both Gastrocnemius muscles the shortest bundles are obtained from the proximal third of the muscle. The longest bundles are found in the distal third, with bundles from the mid-portion occupying an intermediate position.

In order to examine more closely this differential relationship in length of bundle of M. Gastrocnemius, measurements in the growth study were made at five points (A to E as previously described), not along thirds of muscle as for this preliminary work. In addition, a binocular head-band magnifier was used in conjunction with a vernier caliper for dissociating and measuring bundles. By this means experimental accuracy has been increased, and the labour eased enormously.

Measurements of fibre diameter.

Hammond's technique, of measuring the diameter across short lengths of teased out formalin-fixed fibres, appears to be suitable for the purposes of this experiment. However, as cross-sections of muscle can be utilised for the study of fasciculi, fat, connective tissue, in addition to fibre diameter, it was decided to compare the sectional method with Hammond's method.

Three aspects were considered in making this comparison; namely suitability of method for measuring fibre diameter, the number of measurements which affords a representative sample of the fibre population, and the variation of fibre diameter within a muscle.

At the time the observations were made, formalin-perfused muscle was employed for experimental purposes. The tissues used in the growth study were all fixed only after removal from the carcass, five hours post-mortem. Nevertheless, it is believed the value of these preliminary results is not impaired.

Immediately after formalin perfusion of the anaesthetised rabbit, the right Gastronemius medialis and Psoas major muscles were removed. Five slices were cut from each muscle at the equidistant points A to E. After fixation in neutralised ten per cent, formol saline solution, the slices were imbedded in gelatin according to the method of Zwemer (1933). Transverse sections were cut on a freezing microtome at 35μ and mounted in glycerin jelly without staining. Subsequently, the same gelatin-imbedded muscle slices were used for teasing out short lengths of isolated muscle fibres by Hammond's technique.

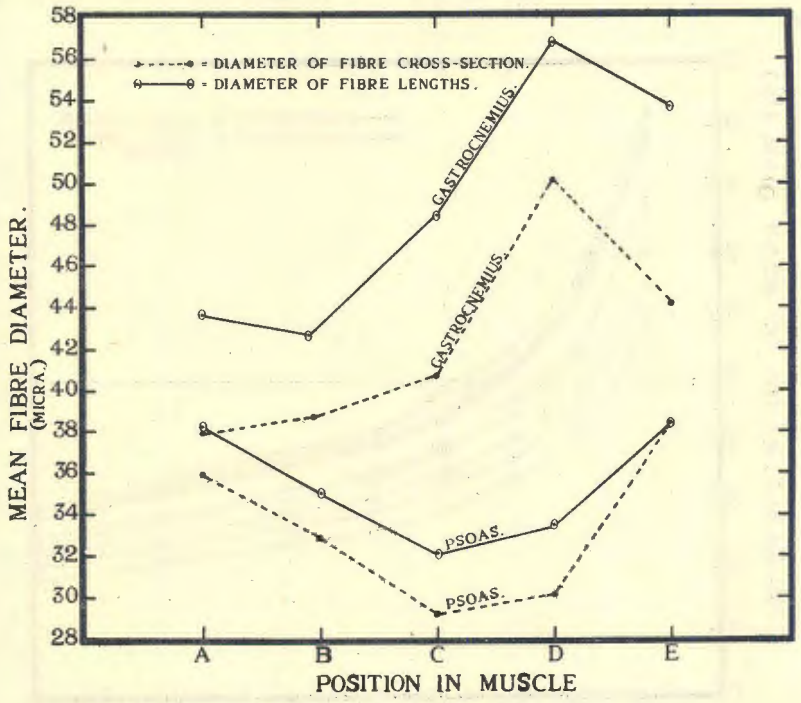

Fig. 2.—Fibre diameter.

Measurement of diameter was made by means of a Zeiss Lanameter at a magnification of 500 times. Both the vertical and horizontal dimensions of the transversely cut fibres were measured in the muscle sections. 250 fibres were measured in this way and the mean diameter determined. In the case of the teased preparations 250 measurements were also made, but only in terms of the diameter across loose fibres.

Each group of 250 measurements was randomised to give two selections of 125, two of 100, three of 75, five of 50, and ten of 25 measurements each. These data were analysed statistically at the five per cent. level of probability to assess the relative value of the two methods.

Discussion.

The diameter of fibres at points A to E, as well as the statistical constants, for both the Gastrocnemius and Psoas muscles are given in Appendix Tables A, B, C and D.

Fibre diameter along the length of the muscle is depicted in Figure 2. In M. Gastrocnemius, there is little difference in fibre diameter at points A and B. Thereafter, there is a progressive increase to C, and to D. Near the tendinous insertion of the muscle, the diameter decreases from D to E. A well-defined gradient exists within the muscle. The smallest fibres are found near the origin of the muscle, and the largest nearer to the insertion. In M. Psoas, however, the thinnest fibres are found about the middle of the muscle, and the thickest fibres at both ends of the muscle. Furthermore, the Psoas fibres are roughly only two-thirds as thick as those from M. Gastrocnemius. Thus the architecture is markedly different in the two muscles.

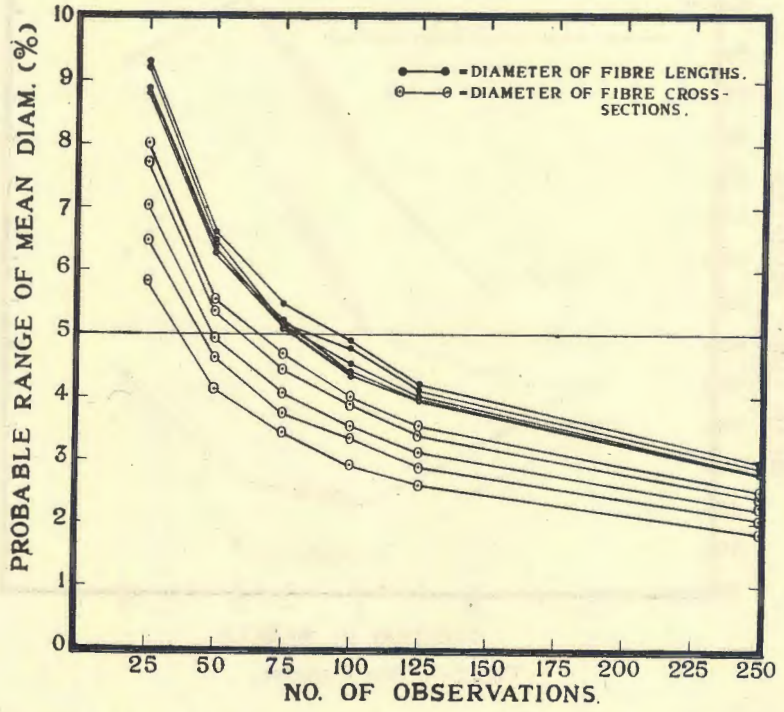

Fig. 3.—Percentage probable range of mean fibre diameter of *M. Gastrocnemius.*

Although the curves for fibre diameter obtained by Hammond's method are nearly parallel to those obtained from cross-sections, the diameter of the teased fibres is greater at all points. The reason is obscure. If the muscle fibres tend to be oval rather than circular, it is possible the teased out fibres settle on the longer axis, which is then always measured. Other factors may be the application of the coverslip to the slide, and the subsequent setting of the mounting medium. Both may tend to flatten the

teased fibre and produce an apparent thickening. This difference in diameter is an indication that care must be taken in mounting the coverslip, so as not to exercise undue pressure on the fibres comprising the specimen.

In Table 4 and Figure 3 are considered the relationship between the number of measurements and the percentage probable range of the mean diameter. As the number of observations making up a group is increased, the probable range of the mean diameter is decreased; markedly from groups of 25 to 50, less pronounced from 50 to 75, and in more gradual manner for the larger groups of 100, 125 and 250 observations.

TABLE 4.

Probable range of mean fibre diameter. Analysis of variance calculated at the 5 per cent. level of probability for selections of 250, 125, 100, 75, 50 and 25 measurements each, Rabbit No. 16.

Muscle.	Number of Measurements.					
	250.	125.	100.	75.	50.	25.
	%	%	%	%	%	%
Right Gastroc. (Cross-section).						
Site A	2·21	3·13	3·55	4·06	4·93	7·03
Site B	1·85	2·61	2·92	3·43	4·13	5·83
Site C	2·40	3·38	3·88	4·44	5·35	7·71
Site D	2·50	3·54	4·00	4·68	5·54	8·01
Site E	2·06	2·90	3·34	3·73	4·64	6·48
MEAN	2·20	3·11	3·54	4·07	4·92	7·01
Right Gastroc. (Hammond's Method).—						
Site A	2·97	4·17	4·91	5·48	6·62	9·30
Site B	2·80	3·95	4·36	5·11	6·27	8·89
Site C	2·79	3·95	4·40	5·22	6·26	8·81
Site D	2·89	4·10	4·76	5·17	6·48	9·23
Site E	2·83	4·00	4·52	5·13	6·37	8·81
MEAN	2·85	4·03	4·59	5·22	6·40	9·01
Right Psoas (Cross-section).—						
Site A	2·18	3·09	3·37	4·05	4·85	6·96
Site B	2·10	2·96	3·25	3·84	4·68	6·56
Site C	2·48	3·50	3·90	4·60	5·48	7·85
Site D	2·62	3·71	4·16	4·83	5·89	8·38
Site E	1·65	2·33	2·69	3·01	3·68	5·18
MEAN	2·21	3·12	3·47	4·07	4·92	6·99
Right Psoas (Hammond's Method).—						
Site A	2·51	3·54	3·91	4·56	5·63	7·95
Site B	2·83	4·01	4·25	5·22	6·37	8·99
Site C	3·41	4·82	4·96	6·13	7·48	10·62
Site D	2·72	3·86	4·41	5·06	6·09	8·67
Site E	2·38	3·37	3·79	4·51	5·33	7·46
MEAN	2·77	3·92	4·26	5·10	6·18	8·74

In the cross-sections, the range exceeds five per cent. for the 25 and 50 groups, but is consistently below the five per cent. level for the groups of 75 measurements, and shows a further slight progressive decrease for the 100, 125 and 250 groups. In the teased preparations, the probable range falls below five per cent only for 100 measurements, as compared with 75 for the cross-sections. However, this reduction in measuring cross-sections is not real, as 150 readings (75 horizontal plus 75 vertical) were taken to obtain the mean cross-sectional diameter for 75 fibres.

In addition, whereas the probable range for the cross-sections varies widely from specimen to specimen, it is relatively constant for the teased fibres. Figure 3 clearly depicts this variability. Such variability may be explained by the experimental difficulty of cutting a truly transverse section. Although care is exercised in aligning the fibres, a reproducible degree of cross-sectioning cannot be obtained in different specimens. This technical difficulty falls away when fibres are teased out according to Hammond's method.

An assessment of the number of fibres which will afford a representative sample largely depends on the degree of accuracy required. For the purpose of this experiment, a measurement of diameter was considered sufficiently accurate, for which the probable range of the mean diameter does not exceed five per cent. This standard of accuracy is achieved by measuring the diameter of at least 100 fibres teased from the muscle specimen. Such a sample is representative of the fibre population and affords reasonably accurate results.

In another investigation of fibre diameter, 500 measurements were made of teased fibres from a number of different specimens. The analysis of these results is presented in Table 5. As the results are essentially similar to those described above, these data are not considered in detail. It suffices to state, these additional calculations confirm the finding that the mean diameter of 100 teased fibres has a probable range of not more than five per cent.

TABLE 5.

Probable range of mean fibre diameter. Analysis of variance calculated at the 5 per cent. level of probability for selections of 500, 250, 125, 100 and 75 measurements.

Muscle.	Number of Teased Fibres Measured.				
	500.	250.	125.	100.	75.
Gastrocnemius—	%	%	%	%	%
Sample 1	2·08	2·99	4·12	4·58	5·31
Sample 2	2·03	2·84	3·97	4·44	5·12
Sample 3	2·21	3·15	4·56	5·00	5·66
Sample 4	2·03	3·03	4·26	4·54	5·30
Sample 5	2·06	2·83	3·96	4·72	5·33
Sample 6	1·98	2·78	3·73	4·59	4·87
MEAN	2·07	2·94	4·10	4·65	5·27

As there is no doubt that Hammond's method of measuring teased fibres is preferable to a sectional method, his technique was applied throughout the growth study. Mean diameter was always calculated from measurement of 100 fibres. In making the selection at random, the method used for wool fibres by Bosman and van Wyk (1939) was followed.

Measurement of texture of muscle.

In order to obtain a measure of muscle texture it is necessary to calculate how many fibres constitute a muscle bundle, as well as the average thickness of these individual fibres.

The fibres were counted in a large number of bundles in each of several muscles, utilising the transverse sections prepared for that purpose as described. These data were subjected to statistical analysis by means of artificial stratified sampling. It was concluded that the error made by using five to ten bundles in a muscle will, on the whole, be the same as that obtained by using twenty bundles. To remain on the safe side, it was decided to select twenty random bundles from each muscle, to afford an average count of the number of fibres constituting the muscle bundle. Hence all calculations in this study are based on an average value obtained from twenty bundles.

(2) *Treatment of data.*

In order to afford a basis of comparison it is necessary that the experimental data be arranged in groups. Either age or live-weight of the animals affords a method of classification. However, there are indications, both on practical and on theoretical grounds, that age is less satisfactory than weight as a standard of comparison.

In Figure 4 a diagram is given representing both age and live-weight of the animals utilised for this experiment. A glance indicates the variability of the age of animals comprising any one group. Between the groups, there is no clear demarcation evident on an age basis, except for the two final groups. By comparison, greater uniformity of live-weight of animals exists within each group. Moreover, there is a clear spacing of all the groups except for the mature animals, as is to be expected.

In order to arrive at simpler indications of the growth processes there appears to be little doubt that the data should be grouped according to weight of the animal. It is to be emphasised, however, that this will result in the two final groups being plotted very closely adjacent in the curves illustrating growth changes. This is true in terms of body weight (3017–3072 Gm.). However, the last group was derived by holding these animals for a period of six months after they had attained a live-weight of 3,000 Gm., so that these rabbits are considerably older than the preceding group.

The data were grouped in twelve groups according to the average live-weight of the rabbits composing each group. Fisher's (1941) " Analysis of Variance " method was used to test the means for each of these groups, the Z test being employed to determine the existence of significant differences,

while the significantly differing groups were picked out by means of the t test. In comparing relative values within the muscle, the means for the various sites (A, B, C, D, E,) were calculated in each group, and these were tested in similar manner.

As standards of significance were regarded the values of Z and t, when $P = 0{\cdot}05$ (i.e. 5 per cent. probability), and when $P = 0{\cdot}01$ (i.e. 1 per cent. probability). X indicates a positive result at the former level (which already indicates definite significance), while a similar result at the higher level of significance is indicated by X X.

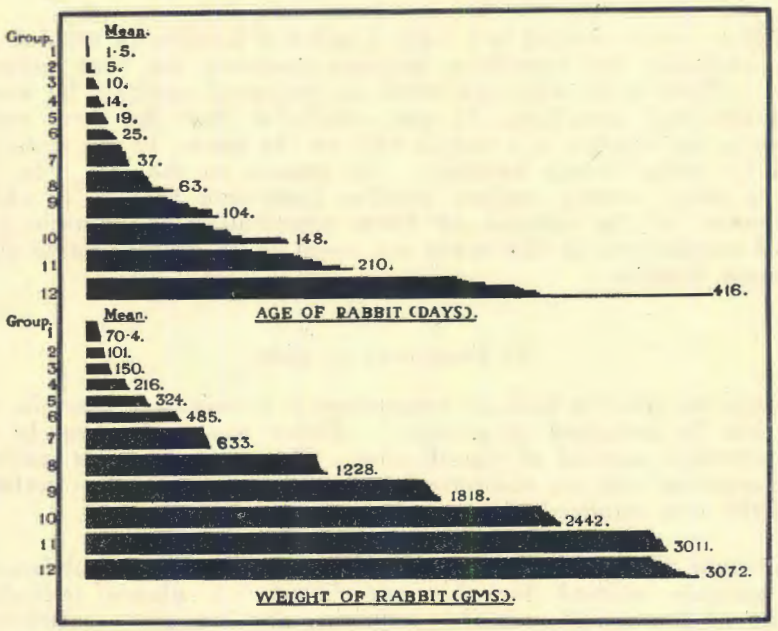

Fig. 4.—Age and live-weight of the experimental animals. Graph compiled from data in Appendix, Table E.

The complete data are shown in a series of tables in the Appendix. For the graphs, smoothed values of the observed mean values were calculated by means of the method of least squares. Smooth curves constructed in this manner are employed in the figures, but the distribution of the actual observed means about the curves is also plotted. Only the observed means for the sites and groups are employed in the tables. In these tables are also indicated the number of each group, the class (or description), and the number of rabbits in each group. Then the mean for the site or group is stated, and the results of the tests for significance. For the intra-muscular analysis (i.e. sites), the results of these tests are given in two columns, the first indicating the results when each site is tested against the one immediately preceding, while the second column shows the results of testing each site against the first site (i.e. " A "). For the inter-muscular analysis, (i.e. groups), the second column is omitted as it has little application.

Huxley's law of simple allometry has been used throughout to express the relation of the measurement of the part to the measurement of the whole, in this case the body-weight of the rabbit. This is written:—

$$y = bx^{a} \quad \ldots \quad (1)$$

where y = measurement of the part,

x = measurement of the whole,

b = a constant,

a = the equilibrium constant.

or, $\log. y = \log. b + a \log. x \quad \ldots \quad (2)$

First, the individual observations were translated to logarithmic form in order to test the linear relationship in logs. For purposes of computation expression (2) was formulated as $\hat{Y} = B + AX \quad \ldots \quad (3)$

where $\hat{Y}$ = best estimate of $Y + \log. y$,

$X = \log. x$,

$B = \log. b$,

A = best estimate of a.

The plotting of log. y against log. x did not result in a straight-line distribution for certain measurements. Hence a logarithmic parabola was fitted to all measurements as a first empirical extension of the allometric formula, thus—

$$\hat{Y} = B + AX + CX^{2} \quad \ldots \quad (4)$$

as equivalent of

$$\hat{y} = bx^{a}\, 10^{c \log.^{2} x} \quad \ldots \quad (5)$$

or, $$\hat{y} = bx^{a + c \log. x} \quad \ldots \quad (6)$$

By means of the method of least squares the equation was fitted to the data. From the anti-logarithms the corresponding expected values of y were determined for the series of values of body weight x. In this way smooth muscle—body-weight curves were constructed for the data.

As the same set of body-weights was used throughout the experiment Fisher's (1941) technique was used to avoid solving the simultaneous equations afresh on each occasion. By introducing Waugh's (1935) method of solving the equations further simplification was possible. After obtaining the numerical values of the constants B, A and C, the last-mentioned was tested for significance. If insignificant, the last term in (4) was omitted and the necessary corrections applied to A and B.

The instantaneous rate of increase relative to body-weight was also determined by mathematical differentiation of the allometric formula, or expression (4), the formulae becoming respectively

$$\frac{dy}{dx} = A \frac{\hat{y}}{x} \quad \ldots \quad (7)$$

and $$\frac{dy}{dx} = (A + 2CX) \frac{\hat{y}}{x} \quad \ldots \quad (8)$$

CHAPTER IV.—OBSERVATIONS.

(*a*) Growth and development of the rabbit.

(Literature: Pages 335 to 337.)

Animal growth and development fall outside the scope of this investigation, except with reference to the basal study of muscle morphology. Hence,animal growth is only considered insofar as it provides a background of the rabbits used for the observations concerning muscle growth and development.

Live-weight with increasing age, and weekly rate of growth as measured in grams per week increase, are shown in Figure 5. These have been calculated from the average columns of Table 6.

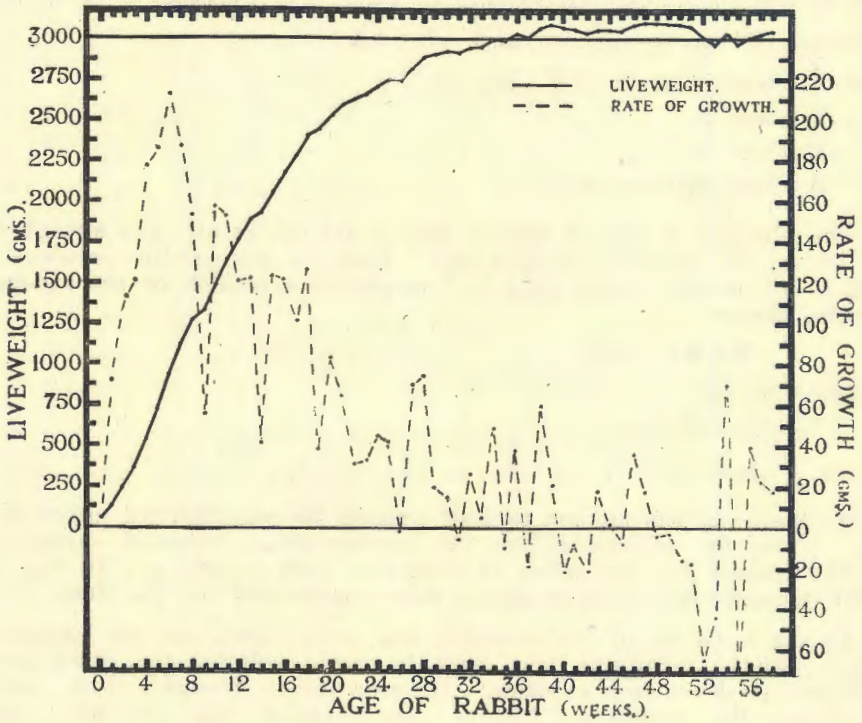

Fig. 5.—Live-weight growth and rate of growth.

Live-weight growth follows the conventional pattern determined for various species. At thirty-four weeks of age the live-weight first reaches a level of 3,000 gm. followed by fluctuation up to a maximum of 3,104 gm. at forty-seven weeks. Thereafter, live-weight fluctuates until fifty-eight weeks of age, when the weight recorded is 3,038 gm. As 3,000 gm. was estimated to be the approximate upper limit of live-weight, it is of interest to see that this agrees with the data compiled from the experimental animals (mean live-weight from thirty-four to fifty-eight weeks is 3,041 gm.). Attention must be directed to the decreasing number of rabbits towards the end of the growth curve. There are less than ten individuals from thirty-seven weeks. This reduction in number of animals should be taken into consideration in evaluating the data, especially near the end of the growth period.

TABLE 6.

Average weight of rabbits.

Age. (Weeks).	No. of Rabbits.	Weight (Gm.).	Weekly gain. (Gm.).	Age. (Weeks).	No. of Rabbits.	Weight (Gm.).	Weekly gain. (Gm.)
Birth.....	50	61	—	30.....	15	2925	15
1.....	63	133	72	31.....	15	2919	−6
2.....	54	241	108	32.....	13	2945	26
3.....	41	362	121	33.....	11	2950	5
4.....	37	540	178	34.....	10	2999	49
5.....	33	726	186	35.....	12	2989	−10
6.....	33	939	213	36.....	10	3027	38
7.....	25	1126	187	37.....	9	3008	−19
8.....	19	1280	154	38.....	9	3068	60
9.....	24	1336	56	39.....	9	3089	21
10.....	23	1494	158	40.....	9	3067	−22
11.....	30	1647	153	41.....	8	3059	−8
12.....	34	1768	121	42.....	9	3038	−21
13.....	31	1891	123	43.....	10	3056	18
14.....	33	1933	42	44.....	8	3058	2
15.....	31	2057	124	45.....	10	3050	−8
16.....	27	2179	122	46.....	9	3086	36
17.....	28	2281	102	47.....	10	3104	18
18.....	22	2408	127	48.....	10	3100	−4
19.....	26	2447	39	49.....	9	3097	−3
20.....	23	2531	84	50.....	10	3081	−16
21.....	22	2596	65	51.....	9	3064	−17
22.....	21	2627	31	52.....	8	2999	−65
23.....	19	2660	33	53.....	6	2955	−44
24.....	22	2705	45	54.....	7	3025	70
25.....	19	2747	42	55.....	6	2957	−68
26.....	21	2745	−2	56.....	6	2997	40
27.....	18	2815	70	57.....	5	3020	23
28.....	16	2890	75	58.....	6	3038	18
29.....	16	2910	20				

Rate of growth increases up to about the sixth week of life, rising from a live-weight gain of 72 gm. in the first week to 213 gm. in the sixth week. After this the growth rate decreases. A negative quantity (−2 gm.) is recorded at twenty-six weeks. Thereafter, until the fifty-eighth week, rate of growth fluctuates with roughly alternating periods of slight gain or loss of weight.

(*b*) GROWTH AND DEVELOPMENT OF MUSCLE.

1. *Weight.*

(Literature: pages 337-338.)

The mean muscle weights for each of the twelve groups are presented both in tabular and in graphic form (Table 7 and Figure 6).

At birth the Psoas muscle is twice as heavy as M. Gastrocnemius. This difference becomes increasingly more marked as the animal increases in weight, so that in the mature animal M. Psoas is approximately four times heavier than M. Gastrocnemius. Significance is not shown for the small cumulative increases in the earlier groups. All increments are, however, significant in M. Gastrocnemius from 320 gm. live-weight onwards, except for the mature group, and in the Psoas muscle from 600 gm. live-weight until maturity.

TABLE 7.

Weight of muscle.

Groups of Rabbits.		No. of Rabbits.	Mean Weight M. Gastroc.	Sig. Test (W. Prec. Group).	Mean Weight M. Psoas.	Sig. Test (W. Prec. Group).
No.	Class.					
			Gram.		Gram.	
1.....	Birth.....	10	0·048	—	0·109	—
	Gram.					
2.....	100	5	0·056	N.S.	0·156	N.S.
3.....	150	5	0·117	N.S.	0·268	N.S.
4.....	220	5	0·226	N.S.	0·431	N.S.
5.....	320	5	0·424	N.S.	0·870	N.S.
6.....	480	5	0·747	X	1·564	N.S.
7.....	600	10	1·00	X	1·95	N.S.
8.....	1200	10	1·90	XX	4·60	XX
9.....	1800	10	2·95	XX	8·60	XX
10.....	2400	10	3·71	XX	11·55	XX
11.....	3000	10	4·64	XX	14·79	XX
12.....	Mature....	10	4·72	N.S.	16·13	XX

Doubt may be expressed as to why the weight increments do not show significance in the early stages. Although a well-marked live-weight difference exists in the animals from which these muscles were derived, it is known that the major portion of the increase in weight of the young animals may be attributed to the early developing tissues and systems, e.g., skin, bone, head, feet. By comparison with these tissues and organs, muscle makes a greater proportion of its growth later in life.

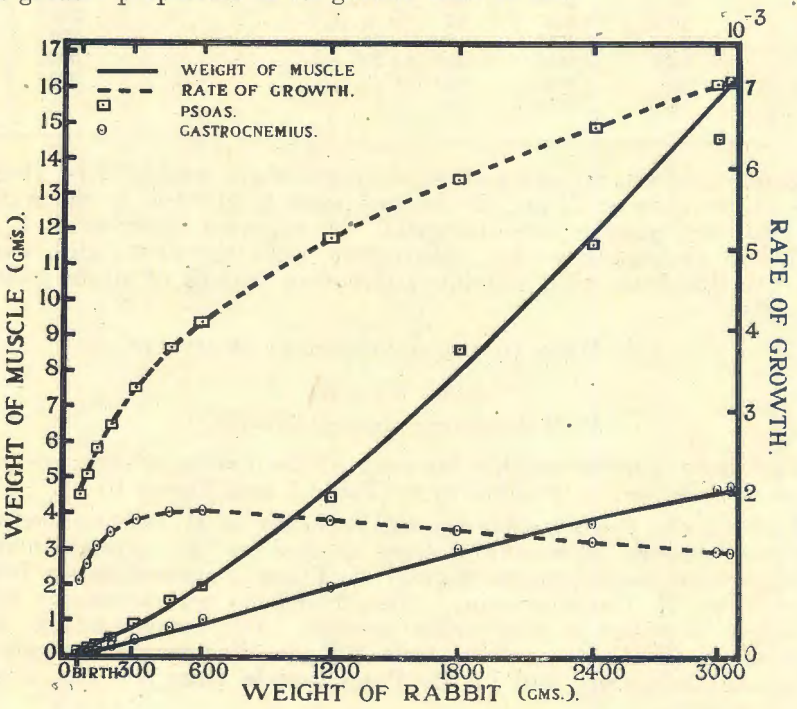

Fig. 6.—Weight of muscle.

Having demonstrated this increase in mass, it is of interest to consider the rate at which the additional muscle substance is accumulated (Table 8 and Figure 6).

It is seen that the rate of growth* of M. Gastrocnemius increases sharply until 220 gm. live-weight. After a subsequent more gradual rise until 600 gm., the growth rate then subsides slowly until 3,000 gm. live-weight, and shows a negligible decrease for the last group.

In the Psoas muscle there is a steady increase in the rate of growth, making for great dissimilarity between the two curves. At birth, the rate of growth of M. Psoas is approximately twice as great as that for the Gastrocnemius muscle. From this point the rate shows a steep upward trend until 600 gm. live-weight. Unlike M. Gastrocnemius, which subsides at this stage, the Psoas growth rate continues to increase until 3,000 gm. live-weight, although in a more gradual manner than in the initial stages. Even in the last group there is a slight final increase. Thus, in the mature animal the rate of growth of the Psoas muscle is more than five times that of M. Gastrocnemius.

TABLE 8.

Rate of growth of muscle weight.

Group.	M. Gastrocnemius.	M. Psoas.	Group.	M. Gastrocnemius.	M. Psoas.
Birth.....	0·000,920	0·001,942	Gram.		
Gram.			600	0·001,782	0·004,116
100	0·001,128	0·002,214	1200	0·001,687	0·005,149
150	0·001,338	0·002,529	1800	0·001,540	0·005,881
220	0·001,515	0·002,861	2400	0·001,396	0·006,499
320	0·001,669	0·003,282	3000	0·001,282	0·006,975
480	0·001,762	0·003,761	Mature.....	0·001,270	0·007,020

Discussion.

It is evident that the mode of growth is different in these two muscles. Whereas M. Gastrocnemius is an early developing muscle making a great proportion of its growth early in life, the Psoas muscle achieves maturity late in the lifetime of the animal. Until this growth has been analysed in terms of the muscle dimensions (length, width, depth), it cannot be decided what share can be attributed to each of these characters.

2. *Length.*

(Literature: pages 338–339.)

Length is considered in Table 9 and Figure 7.

M. Psoas is appreciably longer than M. Gastrocnemius at birth. Throughout the growth of the animal this difference becomes accentuated, so that M. Psoas is roughly ten centimetres longer than the Gastrocnemius muscle at maturity. Nevertheless, the relative proportions are more or less the same throughout the life of the animal.

* Rate of growth indicates the instantaneous rate of increase as determined by the formula on page 367.

TABLE 9.

Length of muscle.

Groups of Rabbits.		No. of Rabbits.	Mean Length M. Gastroc.	Sig. Test (W. Prec. Group).	Mean Length M. Psoas.	Sig. Test. (W. Prec. Group).
No.	Class.					
			Cm.		Cm.	
1.....	Birth.....	10	0·99	—	2·72	—
	Gram.					
2.....	100	5	1·08	N.S.	3·58	N.S.
3.....	150	5	1·50	XX	4·30	N.S.
4.....	220	5	2·04	XX	5·16	N.S.
5.....	320	5	2·50	XX	6·62	XX
6.....	480	5	3·00	XX	8·04	XX
7.....	600	10	3·27	X	8·85	N.S.
8.....	1200	10	4·12	XX	10·17	XX
9.....	1800	10	5·00	XX	12·44	XX
10.....	2400	10	5·48	XX	14·50	XX
11.....	3000	10	5·93	XX	15·82	XX
12.....	Mature....	10	6·15	X	15·96	N.S.

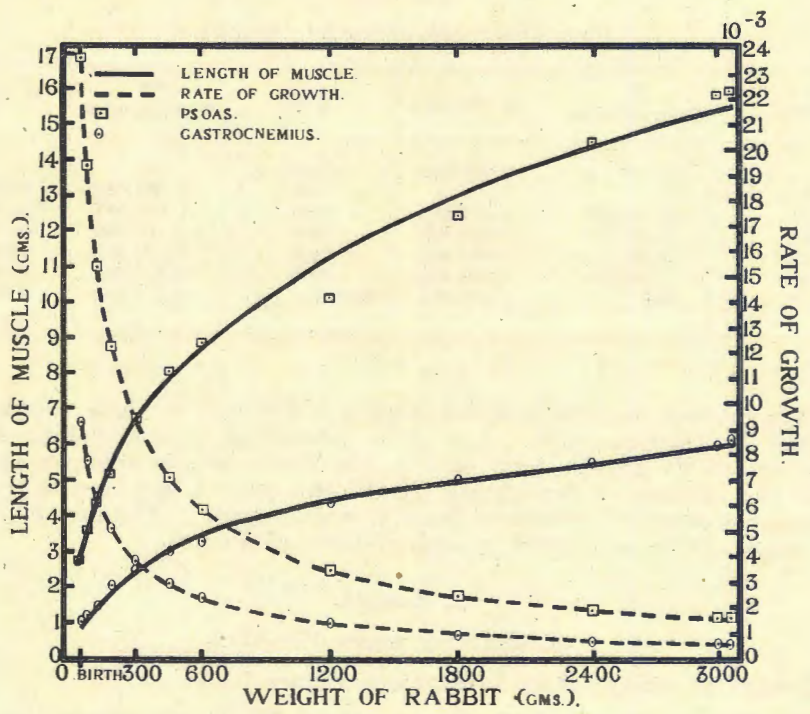

Fig. 7.—Length of muscle.

During each stage of growth from 100 gm. live-weight onwards, significantly differing amounts are added to the length of M. Gastrocnemius. Increments accruing throughout growth of M. Psoas do not show the same regularity of significance. In the earlier stages up to 220 gm. live-weight at 600 gm., and in the mature rabbit the variations show a negative result. This is probably partly explained by the inherent difficulty of measuring the