DIFFERENTIATION OF MUSCLE FIBERS DURING GROWTH AND DEVELOPMENT*

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Figure 1 clearly illustrates that muscle is composed of more than one fiber type. Two considerations are relevant: first, the fiber types have distinctly different properties, and second, the gross characteristics of the muscle are dependent on the proportion of fiber types present. (Moody and Cassens, 1968). The minimum number of fiber types is two, with the red fiber being rich in oxidative enzymes and poor in phosphorylase while the white fiber is poor in oxidative enzymes but rich in phosphorylase. However, there is a range in properties from red to white, as there is with most biological phenomena, and various authors have devised various schemes of classification of fiber typing. Suffice it here to say that fibers intermediate in some property, between red and white, are well recognized. We shall confine ourselves to the use of red or type I fibers, white or type II fibers and intermediate fibers. Beecher (1966) described the complex but descriptive terminology that has evolved in regard to synonyms for fiber types in striated muscle. White fibers have been called agranular, bright, light, fast, large pale, tetanic, twitch, protoplasma reichen and Fibrillenstruktur whereas red fibers have been referred to as granular, dark, slow, small, tonic protoplasma-armen and Felderstruktur. The reader is referred to review articles for a complete account of the histological, biochemical and physiological properties of red and white muscle (Needham, 1926; Denney-Brown, 1929; Cassens and Cooper, 1969).

Muscle fiber differentiation is the topic of this manuscript. Denney-Brown (1929) was aware of the fact, some years ago, that the properties of muscle changed as the animal developed from newborn to adult. He wrote that each muscle fibril, in its primitive form, is packed with granules and the process of contraction is extremely sluggish and delayed; early in the extra-uterine life of the kitten, fiber differences begin to occur. The first of these differences observable is the loss of granular opacity in some fibers. This process rapidly progresses and muscle becomes a mixture of clear and dark fibers. Today, some years after Denney-Brown spoke of such changes in fiber characteristics, we are well informed of the histochemical, physiological and biochemical changes that occur in muscle fibers from foetal stages to the adult. This information will be reviewed and then a resume of our findings on pig muscle will be presented since the members of this organization are interested in aspects of muscle from domestic animals that affects its use as a food.

* This contribution was prepared during the term of support by Public Health Service Grant No. UI-00266-10 from the National Center for Urban and Industrial Health.
The study of differentiation is important so that normal development is catalogued; this allows the researcher to establish when a pathological process may have started (Dubowitz, 1965a). We have been intensely interested in the process of differentiation in pig muscle because Cooper et al. (1969c) demonstrated a possible association between fiber type characteristics and the development of pale, soft, exudative (PSE) muscle. A knowledge of fiber type differentiation should therefore be meaningful to an understanding of when, in the life of the pig, the stage is set for the PSE condition.

**HISTOCHEMICAL STUDIES**

Beckett and Bourne (1958) studied succinic dehydrogenase in biceps brachii, gastrocnemius, tibialis anterior and rectus femoris muscles of goat. Succinic dehydrogenase activity increased during foetal growth but never reached adult levels; the differentiation between light and dark fibers became apparent at about 110-120 days (term is 150 days). They also found little size difference between light and dark fibers. It is interesting in view of some considerations of innervation to be dealt with later, that a moderate cholinesterase concentration was found in 48 day foetus and adult levels were reached by 64 days.

Dubowitz (1963) found in full term human infants that muscle was differentiated at birth and that fibers were rounded and arranged in bundles. Fibers rich in phosphorylase were of slightly greater diameter than those rich in oxidative enzymes, and ATPase activity correlated well with phosphorylase activity in individual fibers. Muscle was differentiated at birth in guinea pig, rabbit and hamster but the differentiation was most striking in guinea pig where fibers were polygonal in shape and arranged in bundles that resembled adult muscle. In rabbit and hamster muscle, the differentiation was less marked, the fibers tended to be rounded and were not so clearly grouped into bundles. Fibers were not differentiated at birth in rat muscle but the differentiation usually occurred between 7 and 10 days post-partum. There were some areas of focal differentiation apparent at 2-3 days. The process varied from one group of muscle to another and was noticeable with the ATPase reaction before it was with some of the other histochemical tests. He thought that differentiation was influenced by nutrition and general growth rate; rats from smaller litters tended to be larger and more mature and muscle showed earlier maturation. Dubowitz (1963) also suggested that the difference in muscle maturation for these animals could be accounted for by their length of gestation and degree of general maturity at birth. The guinea pig (68 day gestation) has a more mature fur at birth and is much more mobile than the other animals. The rabbit (30-32 days gestation) has well developed fur and is more active than the rat (21 day gestation). The hamster, although having a shorter gestation (16-19 days) than the rat, also appears more active at birth and its fur develops more rapidly.

Wirsen and Larsson (1964) conducted an experiment on the muscle of mice (14-21 days gestation, new born, 1 day old and 7 days old). Succinic dehydrogenase and lipid staining did not reveal any distinct differences among fiber types before birth and peroxidase activity, interpreted as due to myoglobin, was not demonstrable until after birth. On the 16th gestational day some strongly phosphorylase positive myotubes (primary fibers) were observed. Also apparent were some smaller fibers, in close contact with the primary fibers but with a weak phosphorylase reaction (secondary fibers). The 17th and 18th
gestational day was generally characterized by rapid development of secondary
fibers which in certain muscle groups had become as large or larger than
primary fibers. On the 19th gestational day, a third fiber type, the tertiary
was observed and it was practically phosphorylase negative. At this stage
the fibers were becoming more closely packed and were changing from rounded
to polygonal shape. In newborn and 1 day old mice, all three fiber types
were seen in several muscles. The authors concluded that during foetal
development there is a gradual transition from glycolytic to presumably
oxidative metabolism as reflected by the strong phosphorylase reaction of
primary fibers compared to the moderate staining of secondary fibers and the
very weak phosphorylase reaction of tertiary fibers. They interpreted their
results as not supporting the direct correlation between gestation length and
maturation of muscle as proposed by Dubowitz (1963). They thought that the
three foetal populations (primary, secondary and tertiary) could be tenta-
tively classified as white, intermediate and red of adult musculature and
proposed that the 3 types develop as 3 distinct populations.

Dubowitz (1965a) restated his views about developing animal muscle
in a later publication; there was a striking difference in maturation of
skeletal muscle in different species of animals. He thought there was a
correlation between differentiation and general maturity of muscle at birth
and also with length of gestation. He studied guinea pig muscle in more
detail by including some foetal stages; muscle from newborn guinea pig
resembled adult muscle and was fully differentiated into type I and type II
fibers. In foetus of 35 to 40 days gestation, most muscle showed complete
uniformity of fibers with NADH-diaphorase, apart from some variation in
overall stain of some whole bundles as compared to others. In occasional
muscles there was some variation between individual fibers within a bundle.
With phosphorylase and ATPase, in contrast, most muscle showed subdivision
of fibers into strongly and weakly reacting ones, but did not give the clear
cut checkerboard pattern of mature muscle. At 50-55 days gestation, all
muscles revealed a clear cut differentiation into type I and type II fibers
with various enzyme reactions and showed the checkerboard pattern of mature
muscle.

Dubowitz (1965b, 1966) also published further extensive information
on fiber differentiation in developing human muscle. In all infants and
children (2 weeks - 8 years) the muscle showed a checkerboard pattern with
subdivision into at least 2 fiber types as in adult muscle. Biopsy sampling
gave better results than necropsy. The muscle from newborn full term infants
had a pattern of differentiation similar to that of older infants. Newborn
premature infants showed a similar pattern of differentiation into fiber types,
but results were less consistent than in full term infants. Most newborn
infants had an approximately equal proportion of type I and type II fibers.
Foetuses were divided into 2 groups on the basis of size; larger foetuses
were 20-26 weeks old and smaller foetuses were 12-20 weeks old. The larger
foetuses showed 2 groups of fibers with the NADH-diaphorase reaction--one
very intense and the other moderate. Phosphorylase and ATPase results were
not as consistent but a number showed the exact reciprocal to the NADH-
diaphorase reaction. The above pattern was not present in the smaller foetuses.
Some muscles were completely uniform and some showed variation from bundle to
bundle, but all fibers within a bundle were uniform. Some showed weakly or
strongly reacting fibers for a particular enzyme and did not form the checker-
board pattern, but occurred in clusters. In serial sections in a number of
instances, fibers with a strong activity for one enzyme were also found to
have a high content of other enzymes so that they did not correspond to type I or type II fibers of mature muscle. In these younger foetuses the fibers were in widely separated small groups and not in compact bundles. He divides developing human muscle into 3 phases. Phase I represents early foetal life to about 20 weeks. There is no clear cut division into fiber types and certain fibers cannot be correlated with type I or type II of mature muscle. Phase II represents 20-26 weeks gestational age and there is a clear cut division into 2 fiber types. However, type I fibers comprise only a very small proportion of the total. Phase III represents about 30 weeks to full term. Differentiation is similar to adult muscle with approximately equal proportion of type I and type II fibers.

Developing human skeletal muscle has also been examined by Fenichel (1966) during the gestational age 5-20 weeks. Fiber typing could not be accomplished during the period 5-8 weeks: the mitochondrial reactions were uniformly intense, and the total quantity of available myofibrils was insufficient to allow conclusions to be drawn from the myosin ATPase activity. Light and dark myotubes could be distinguished with the ATPase reaction during the period 8-10 weeks. Type II fibers (dark with ATPase and average 15-20 μ in diameter) were more numerous than the smaller type I fibers (light) that averaged 5-10 μ diameter. Mitochondrial enzyme activity continues to be diffusely intense in fibers undergoing new filament formation which precludes their use for fiber typing. Tye II fibers remain larger than type I fibers through the 15th gestational week and many mature myocytes are present which can be typed with mitochondrial enzyme reactions. The muscle has a mature appearance by 20 weeks; the 2 types are about equal in number but their size relationship has reversed with type I being larger than type II. The author concluded that mitochondrial enzyme reactions were of uniform intensity in all muscle fibers during the period of myofibrillary formation and could not be used for fiber typing until the transformation from myotube to myocyte was complete. The inability to use the oxidative enzyme reactions for fiber typing until fibrillar formation is completed probably reflects the uniformly high rate of enzyme activity needed during the early stages of muscle development in the production of new myofilaments. Fenichel (1966) also found ATPase to be the most useful reaction, and he concluded from his results, that the 2 muscle types develop in human embryos as separate populations.

The phosphorylase characteristics of chicken muscle have been studied (Cosmos, 1966; Cosmos et al., 1965). In adult domestic fowl, aerobic slow fibers of the heart give a reaction similar to the red iodine color produced by short chain amylopectins. Anaerobic fast fibers of breast muscle gave a blue iodine color resembling long chain amylose like products in leg muscle, in addition, had fibers with an intermediate range of purple colors. Their tests with embryo breast muscle showed an amylopectin type reaction that indicated the presence of slow aerobic fibers. Differentiation to more specialized fast fibers began during the first week ex ovo when there was a decrease in slow fibers and a concomitant increase in intermediate and fast types. The adult breast muscle was composed mainly of fast fibers. The authors concluded that the development of cells of breast muscle progress from a slow fiber to an intermediate one and finally to the highly differentiated fast fiber characterized by polysaccharides of long unbranched chains.

Chinoy and George (1965) studied the pectoralis muscle of pigeon. They used 15-17 day embryos and ex ovo birds of 1-8, 16, 24 and 40 days. The
distinction between the broad and narrow fibers was clearly defined as early as the 15th day in ovo. The levels of fat and succinic dehydrogenase appeared greater in narrow fibers than broad fibers in 2 day old birds. In 8 day old birds the narrow fibers were more sudanophilic than broad fibers and contained numerous mitochondria. In 8 day old birds, the narrow fibers contained considerably more fat and succinic dehydrogenase than broad fibers in which the mitochondria were fewer and smaller. At 16, 24, and 40 days of age, the narrow fibers were distinctly more sudanophilic and contained greater concentrations of fat, succinic dehydrogenase and lipase than did broad fibers. The authors concluded that the differentiation of fibers into broad white and narrow red takes place in the embryo itself and not during post-embryonic life, at which time there is increased activity of muscle. The 2 types of fiber were easily distinguishable on the 15th day of incubation. Glycogen was depleted after about the 3rd day ex ovo. Fat was then incorporated into the narrow fibers and simultaneously the mitochondrial density and succinic dehydrogenase activity were rapidly increased in narrow but not broad fibers. They interpreted this to mean that the narrow fibers were being prepared for oxidative metabolism while the broad fibers were not.

Germino et al. (1965) used the succinic dehydrogenase technique to study development of skeletal muscle in the chick from the 3rd day of prenatal existence to 60 days after hatching. They found the development of muscle cells to be chronologically parallel up to the 16th day in regard to intensity and distribution of the reaction. During the 17th and 18th days a few fibers appeared that presented only a moderate reaction. These fibers were called intermediates as they were thought to arise from those having an intense reaction—the only ones existing at that time and which were red fibers. During the following days, the succinic dehydrogenase activity of the intermediate fibers decreases even more and they become white fibers. Myofibers are already constituted when differentiation in red, intermediate and white fibers is seen, and it takes place between the 17th and 19th day of embryonic life. The researchers made the following statement about their results: the large amount of succinic dehydrogenase already present in such early stages as the premyoblast expresses the greater energy requirements of the muscle cell when one considers succinic dehydrogenase as an expression of TCA cycle activity. This activity, which is at first perinuclear, would seem to be related to the nucleo protoplasmic transfer of RNA, which is very great in the early stages of growth. The energy requirements in later stages, especially when the reaction spreads all along the sarcoplasm, appears to be related to the muscular contractions which are visible from the 4th and 5th days.

Rebollo and Piantelli (1964) have studied the lipids in chicken skeletal muscle during development. In proximal leg muscles the 2 types of fibers develop sometime after the 12th day. The future straight line red fibers have intermyofibrillary granulations (positive to lipid techniques); similar granulations are found in the future white fibers although in a smaller number. From the 18th day on, an important increase in size of intermyofibrillary granules is observed and 3 types of fibers are distinguishable: red fibers with abundant lipids, white fibers in which lipids are scarce and fibers intermediate in this aspect. However, the fiber number, size and distribution are not yet similar to the adult stage. In pectoral muscle, the lipids disappear on the 14th day. These
fibers undergo a much slower maturation from the histogenic standpoint but mature earlier in regard to distribution of lipids. Morphological features (Rebollo et al., 1963) and lipase distribution (Piantelli and Rebollo, 1967) in developing muscle of chicken have also been reported.

Nystrom (1966) used the succinic dehydrogenase reaction to study differentiation in gastrocnemius, soleus and extensor carpi radialis muscles of 1, 10, 15, 39 day old and adult cats. No differentiation was seen in gastrocnemius of newborn, at 10 days some differentiation was visible and at 15 days it was seen clearly but intermediate fibers were not present. All 3 fiber types were present in 39 day old cats but the muscle still did not wholly resemble adult muscle. No differentiation was present in soleus muscle of newborn. Between 2-7 weeks, some differentiation into more darkly and sparsely staining fibers was evident. After 7 weeks, however, all fibers stained darkly and as in the adult and there was no differentiation into fiber types. The extensor carpi radialis is a muscle of the forelimb and differentiation is present at birth; it has a similar staining pattern to gastrocnemius (white muscle) of adult. Other authors have shown that the radial nerve of the forelimb is more mature than equally distal nerves in the hindlimb of newborn kittens as indicated by diameter of the nerve fibers. Thus, the authors thought it probable that the differentiation mechanism started at a certain stage of maturation in motorneurones. Differences in degree of maturity of motorneurones could also explain the species differences found at birth in histochemical differentiation of muscle.

Fiber differentiation has been studied in rhesus monkeys by Beatty et al. (1967). Succinic dehydrogenase activity was found present in fetuses at 76-78 days, but sections from younger fetuses required longer incubation to demonstrate activity. It was not possible to differentiate fibers into typical small red and large white types in brachioradialis of 90 day fetus. Neither was it feasible to distinguish between brachioradialis and soleus on the basis of fiber type. However, by 120 days, red and white fibers were clearly identifiable and the distribution pattern was similar to that in adult muscle. The 2 red fiber types of the soleus that have been described for the adult cat, rat and rhesus monkey could be seen in the 120 day fetal soleus.

We will comment on 2 recent additions to the literature as a conclusion to this section on histochemical studies. Dubowitz (1968) has published an interesting book on Developing and Diseased Muscle. Much of it is a review or extension of his earlier work but two observations deserve comment. A clear cut dividing line could not be established in rats between the phase when muscle fibers were uniformly positive for all enzyme reactions and the phase of complete differentiation into the adult types. It is a gradual process and the initial occurrence is in random parts of the muscle. Some muscles showed early differentiation with one enzyme reaction and complete uniformity with others. With myosin ATPase, it was possible to recognize isolated darkly staining fibers in some muscles as early as the 1st day of life. Dubowitz thinks that embryologically, all muscle consists initially of one fiber type only and that this corresponds to the type I or red fiber type. The red muscle shows no histological change during maturation, but all the other muscles subsequently differentiate into type I, type II and intermediate fibers. These histological results parallel very closely a number of observations by physiologists on developing muscle that will be described in a subsequent section of this manuscript.
Finally, we will outline the recent extensive histochemical results of Nystrom (1968a) on developing cat muscle. He studied the gastrocnemius (fast white) and crureus (slow red, similar to soleus) in kittens 1-120 days of age and in adults. His A fibers are white or large diameter and poor in oxidative enzymes, C fibers are red and rich in oxidative enzymes and myoglobin and B fibers are intermediate. The main type of fiber in slow red muscle was found not to conform to any of the types present in fast white muscle and is denoted as S type. The fast white gastrocnemius had C, A and B types. Type C stained darkly for succinic dehydrogenase, NADH-TR and lipids but faintly for phosphorylase, glycogen and ATPase. Type A was weak for succinic dehydrogenase, NADH-TR and lipids but strong for phosphorylase, glycogen and ATPase. Type B was seen clearly with succinic dehydrogenase, NADH-TR and lipids and the intensity was close to type A for phosphorylase, glycogen and ATPase. The majority of fibers stained homogeneously for ATPase, succinic dehydrogenase and NADH-TR in crureus muscle. The stain for ATPase was higher than for type C but lower than for type B. A few aberrant fibers in this muscle were very high for ATPase and succinic dehydrogenase but not for NADH-TR. The general type of fiber in slow red was not directly comparable with any of the 3 types in fast white. During post-natal development in newborn kittens the fibers within both slow red (soleus) and fast white (gastrocnemius) appeared fairly uniform except in ATPase section. Different types appeared in both muscles with time, but more so in the gastrocnemius. There was a steady progress in differentiation to the stage of 3 adult fiber types in gastrocnemius. In soleus, however, the tendency to differentiate into various types was soon suppressed and subsequently only one type was present apart from a few aberrant fibers. The gastrocnemius of newborn had similar fibers with succinic dehydrogenase but a slight differentiation into darkly and lightly stained fibers appeared by 10 days. The differentiation was more distinct at 15 days and B fibers began to appear at about 6 weeks. Soleus from newborn also had uniform staining. There was a slight differentiation into pale and light fibers by 2-7 weeks but thereafter the fibers stained uniformly and darkly except for a few aberrant ones. The results with ATPase reaction were different. The gastrocnemius from newborn was distinctly differentiated into light and dark fibers. The soleus showed some differentiation but the amount of light fibers seemed greater. Three types were seen in the soleus at 3 weeks of age but by 6-7 weeks, mainly 1 type was present. Nystrom (1968a) felt that 3 kinds of information are needed to understand the true characteristics of a fiber and its differentiation. The are: (1) the absolute twitch contraction time, (2) the absolute size or conductive velocity of the nerve fiber and (3) the histochemical type.

**PHYSIOLOGICAL STUDIES**

Buller et al. (1960a, 1960b) published 2 papers that stand as classics in physiological studies on red and white muscle. In the first paper (Buller et al., 1960a), they point out that Denney-Brown and some others had demonstrated some time ago that all limb muscles were slow at birth and differentiated into fast and slow types in mammals during the first few weeks after birth. The shorter after-hyperpolarization of motorneurones supplying fast muscles allows their fast frequency of firing which is appropriately related to the contraction time of their muscles—the complimentary conditions apply for slow muscles. Buller et al. (1960a) set about trying to determine whether appropriate matching of motorneurones to muscle was brought about by the motorneurones influencing muscle differentiation.
or vice versa by muscle influencing motorneurones. They recorded the isometric twitch and tetanic response of hind limb muscles of cats over a wide range of ages from 1 day to adult. Responses were compared under standard conditions of temperature and initial tension. Earlier work was confirmed in that all muscles were slow in the newborn, and those muscles destined to be fast attained their adult speed in about 6 weeks. The slow muscles (soleus and crureus) also quicken, but to a lesser extent, over the first 5 weeks; thereafter there is a progressive slowing of these muscles and they approach the adult slow muscle condition in 16-20 weeks. The same results were found with all 3 of the following measurements: (1) time to summit of twitch, (2) time from summit to half relaxation of twitch and (3) interval between stimuli at the tetanic frequency building up to 1/2 the maximal tetanic contraction. The differentiation of fast muscle was virtually unaffected by spinal cord transection or by operative isolation of the lumbosacral spinal cord from all incoming impulses. However, the differentiation of slow muscle was greatly depressed, there being a complete failure of the late phase of slowing so that in a few weeks soleus and crureus became nearly as fast in every respect as normal fast muscles. The authors concluded that the differentiation of slow muscle was largely effected by neural influences exerted from the spinal cord. This conclusion was investigated in subsequent experiments (Buller et al., 1960b) on nerve cross-union in cat hind limbs in order to discover if motorneurones determine the speed of muscles or if the effective influence is in the reverse direction (slow-soleus and fast-flexor digitorium longus were the muscles most often used). When a nerve from fast or phasic motorneurones has been made to innervate a slow muscle, the muscle is transformed to a fast muscle—even in the adult. Likewise, slow or tonic motorneurones convert fast muscles to slow. The transformation of muscle speed was shown not only by the time course of the rising and falling phases of the twitch contraction, but also by the summation of tetanic contractions at various frequencies. The slow or fast muscle with alien innervation had no detectable influence in the reverse direction; i.e., on the motorneurones. The conduction velocity of the motor axons and the time course of the motorneurones after hyperpolarization were both unchanged. These researchers also noted that the muscle transformation fell short of a complete change to the slow or fast type. Isolation of the lumbosacral spinal cord from all incoming impulses, with consequent silence of motorneurones, caused a failure of all transformation by cross-union. Mere transaction of the spinal cord greatly reduced the transformation. They concluded that the neural influence on muscle speed was not exerted by nerve impulses as such. It was postulated that a substance passes down the axons of slow motorneurones, crosses the neuromuscular junction and traverses the muscle fibers transforming them into slow contracting units and maintaining them so. Possibly there is also a substance from fast motorneurones that acts via a comparable pathway to accelerate muscle contraction.

Close (1964) has also studied the extensor digitorum longus and soleus muscles of cat from birth to 14 weeks. At birth, the form of isometric contraction is similar in EDL and soleus muscles. Subsequent development leads to decreases in the contraction times, the times for half relaxation and the twitch/tetanus ratio, whereas there is an increase in the optimal frequency for repetitive stimulation. The force/velocity properties of EDL and soleus are virtually identical at birth; thereafter, the speed of shortening/sarcomere, for any given fraction of the maximum load, increased by 2-1/2 to 3 times in EDL whereas soleus undergoes little or no change in this respect. The author discussed the results in connection with possible
changes in the time course of the active state and the possible dependence of the duration of the active state upon the force/velocity properties.

Nystrom (1968b, 1968c, 1968d) has studied various aspects of innervation in developing cat muscle as it relates to differentiation. One manuscript (Nystrom, 1968b) deals with the characteristics of the motor nerve terminals. True slow red fibers (cold blooded animals, some birds and mammalian extraocular muscle) have a motor nerve terminal referred to as "en grappe" while that in true white fibers is "en plaque." Nystrom (1968) emphasizes that in cat muscle the so-called fast white and slow red are in fact both twitch type muscles. A difference did exist, however, between the gastrocnemius and soleus terminals even though both were of the "en plaque" type. The gastrocnemius end ramifications were wide spreading, long and smooth, but the soleus end ramifications were more tightly packed, wrinkled and fluted in outline. Nerve terminals in 1-7 day old kittens were uniform small rounded discs on the surface, and there was no difference between the gastrocnemius and soleus except that in the soleus the nerve terminal was sometimes larger. The terminal ramifications of gastrocnemius end plates were well established by about 2 months and the soleus had attained a wrinkled appearance and elongated shape. The lower levels of adult size were reached at this stage and the ratio of end plate diameter/muscle fiber size was roughly 1/2 in both soleus and gastrocnemius. If the ratio of end plate size to muscle fiber size is in any way related to the threshold for excitation of muscle fibers then the above results cannot explain the large difference found between the 2 muscles with respect to the post-tetanic twitch potentiation. No systematic variation in structure of the motor nerve terminals was found between muscle fibers of small and large size, in gastrocnemius, that were presumed to represent the histochemical red and white fiber types respectively. He concluded that the structure of the motor nerve terminals and end plate/muscle fiber size ratio cannot explain the difference between soleus and gastrocnemius with respect to the post-tetanic potentiation of twitch tension.

The end plate bound acetyl choline esterase was also studied (histochemically) as a function of postnatal development (Nystrom, 1968c). Staining intensity for acetyl choline esterase appeared to be similar in gastrocnemius and soleus end plates of newborn kittens. However, it increased in gastrocnemius during postnatal development but remained unchanged in soleus. He concluded that the low acetyl choline esterase activity of soleus end plates in adult cats might partly explain the well-known post-tetanic readiness to repetitive discharge found in the soleus. The results obtained with regard to structure of the subneural apparatus and activity of end plate esterase cannot, however, further the understanding of the cause of differences in post-tetanic potentiation of twitch tension following low frequency tetanization between soleus and gastrocnemius in both young and adult kittens.

Nystrom (1968d) also studied the fiber diameter in nerves to slow red and fast white muscles during development. The literature has shown that nerve fibers to small red are smaller than those to fast white; one might expect to find, in the newborn cat, nerve fibers of fairly uniform diameter to future slow red and fast white since contraction times are fairly similar. Nystrom (1968d) found that gastrocnemius and soleus nerve fibers differed inappreciably during the first 10 postnatal days. From then on a distinct difference developed with the gastrocnemius nerve fibers being larger. Results showed consistently smaller fibers in nerves to slow red than to fast white.
In adult cat. In kittens about 2 months old, the efferents of gastrocnemius nerve had become definitely faster than the afferents and they both conducted much faster than the soleus fibers. At this age, as in the newborn kitten, no difference was found between the conduction velocity in efferent and afferent fibers of soleus nerve. A rapid increase occurred in the total number of myelinated fibers in the nerves during the first few weeks of postnatal life and adult values were reached at about 4-6 weeks of age. Nyström (1968) concluded that differences observed between soleus and gastrocnemius with regard to post-tetanic potentiation in young kittens cannot be explained by the slight difference in maturation of their nerve fibers—although with the method used, a difference in the intramuscular branching cannot be excluded.

Goldspink and Rowe (1968) think that the change in speed of muscle during growth is associated solely with the completion of "embryological" differentiation and is in no way relatable to the post-embryological growth and development of the fibers. They also state that increase in speed of muscle during its development (biceps brachii of rat in their case) is due solely to differentiation of the excitatory apparatus of the fiber and is not due to any other mechanism or structural change or alternatively to change in myosin ATPase. We should also point out the system Goldspink uses for morphological description of fibers. He recognizes 3 phases: a small phase (20 μ), interphase (33 μ) and large phase (60 μ) which he presumes correspond to the 3 fiber types.

**BIOCHEMICAL STUDIES**

It is apparent that the biochemical machinery of muscle should parallel the changes that have been shown to occur with histochemical and physiological techniques as muscle develops. We will here review some of the biochemical studies on muscle development.

Latzkovits and Domonkos (1965) studied the effect of postnatal development on carbohydrate metabolism of tonic and tetanic muscles of the rabbit. According to these authors, the best metabolic indicator of the tetanic character of a muscle is the production of aerobic lactate and especially that of aerobic pyruvate, since the processes occur in tetanic muscles and their fibers only. No such process can be observed in tonic muscles. The longitudinalis dorsi was used as a tetanic muscle and abdominal muscles as tonic; they were incubated in Krebs phosphate with air as the gas phase. The metabolism of the skeletal muscles corresponds entirely to that of tonic muscles immediately following birth of the animals. The characteristic metabolism of the tetanic muscle is the result of postnatal differentiation. The authors placed the differentiation of metabolism parallel with the differentiation of muscle function, and before the ability to move independently and locomotor function have developed, tetanic and tonic muscles cannot be dealt with separately or cannot be distinguished.

Cosmos (1966) has studied the development of phosphorylase in developing muscle (superficial pectoralis) of domestic fowl and at the same time has made the same observations in dystrophic muscle. Adult chicken breast muscle has a strong phosphorylase activity histochemically, but in birds with muscular dystrophy the reaction is mixed. She speculated that the diversity of enzymatic response in the various adult muscles merely reflected
stages of development of one common fiber type and there should be a period in development when all fibers give a similar phosphorylase response. With further maturation, some fibers should continue to differentiate to those specialized for specific metabolic function while others continue to grow with no alteration in specific enzyme response. Thus at some period of development, the most highly differentiated fibers must give a phosphorylase reaction similar to that of adult fibers showing minimal enzyme alteration. Cosmos (1966) found that total phosphorylase activity in the embryo was similar in both normal and abnormal muscle. During the early period ex ovo there was a rapid increase of activity that reflected white fiber development. Maximum activity was attained in 6-8 weeks. In dystrophic muscle, the slower rate of development during the first week was followed by a rapid increase during the second week; a change in rate of enzyme development after this period led to a decrease in phosphorylase activity and low levels of the enzyme were maintained throughout maturity. She equated percent active phosphorylase as being phosphorylase a/total phosphorylase. All values were greater than 65-70% from 4-32 days ex ovo which indicated that phosphorylase a was the predominant enzyme in the homogenate. In the embryo, however, all values were low and were taken to mean the relative absence of phosphorylase a at that period. Other evidence on the color of iodine reaction in homogenates is cited to show a stronger reaction as the muscle develops, particularly 14-74 days. To summarize, there was a rapid enzyme increase in breast fibers during early ex ovo life. A 50 fold increase in enzyme activity is seen from hatching to 6 weeks and probably reflects rate of white fiber differentiation. The white fibers are highly differentiated cells adapted to anaerobic functions.

Hartshorne and Perry (1962) studied sarcoplasmic proteins from foetal and adult rabbit muscle. They found generally that the more positively charged protein components, which are readily eluted from diethylaminoethyl-cellulose, are relatively much decreased in sarcoplasm isolated from foetal-rabbit skeletal muscle and from adult heart muscle compared with the amounts present in the corresponding fractions from adult skeletal muscle. More specifically, aldolase activity in foetal skeletal muscle sarcoplasm is comparable with that in adult heart muscle sarcoplasm, but much lower than that in adult rabbit skeletal muscle sarcoplasm. Enzymic activity rises as the foetus develops, and the general conclusion of this comparison is that, at least for the components moving toward the cathode, foetal muscle sarcoplasm is more comparable with that of adult heart muscle than that of adult white skeletal muscle. The sarcoplasm of red skeletal muscle appears to be intermediate between these 2 types. The authors thought that the high concentration of aldolase in adult skeletal muscle sarcoplasm might be expected in view of the well developed ability of that tissue to function anaerobically and furthermore suggested that after birth, aldolase and other proteins of high isoelectric point increase rapidly in amount in response to the increased activity of the skeletal muscle.

Kendrick-Jones and Perry (1967) state that biochemical development in muscle involves the evolution and growth of a contractile system and the development of enzyme systems to convert metabolic fuels to ATP. The contractile system of mammalian striated muscle is very similar among muscle types as to structure and biochemical composition, but the major differences among types are reflected mainly in the non-myofibrillar fraction. These researchers studied AMP-deaminase and creatine phosphokinase. Leg, cardiac
and diaphragm muscles were studied in rabbit. All muscles had low activity in 20-24 day foetus. Cardiac muscle activity remained low throughout to the adult stage. Diaphragm muscle activity increased rapidly 4-5 days before parturition, reaching a maximum before birth, and then fell slowly to the adult level. Leg muscle was constant until 6-9 days after birth and then rose steadily to the adult level by about 14 days (7-8 times increase). This increase in leg muscle AMP-deaminase occurred during the period when young animals began to leave the nest and move about independently. AMP-deaminase increased rapidly in rat leg muscle as the newborn began to move about but in chick and guinea pig leg muscle it rose rapidly before birth and reached the adult level soon afterward. Adenylate kinase closely paralleled the change in AMP-deaminase in all species. Likewise, creatine phosphokinase activity was low in foetal tissue but increased at that stage of development at which activity rose depending on the species and muscle. These researchers thought, from interpretation of the data, that use of the muscle might be a stimulus for rise in specific activity of the enzymes. Therefore, young rabbits were encouraged to move about earlier than usual and in these disturbed litters the creatine phosphokinase activity increased more rapidly and adult values were approached earlier than in control animals. The results are most interesting in view of the fact that AMP-deaminase is most specifically associated with muscle. In rabbit, the muscle activity is 60-100 times greater than in any other tissue. It's significant that there was a good correlation between rapid increase in enzyme activity and time at which contractile activity began to rise rapidly. The results strongly suggested that change in activity was not in response to a systemic stimulus but endogenous to muscle itself. It could be in response to development of nerve system of muscle or frequency of stimuli received; i.e., activity pattern. The fact that animals born at a more advanced stage of development (like guinea pig) have already high enzyme activity fits well with previous histochemical observations (see Dubowitz, 1968). The authors point out that the possibility of activation of enzyme precursors or change in isoenzyme complement to more active enzymic components cannot be ruled out.

The myofibrillar proteins have been considered during development of rat skeletal muscle by Ermine and Schaub (1968). They used myofibrils for assay and found the major changes during the first 3 weeks of foetal life. The Mg"ATPase activity increased about 4 times, whereas Ca"ATPase activity at low ionic strength only doubled. Ca"ATPase at high ionic strength and pH 9.0 increased about 2 times. The low activity of Ca"ATPase of natural actomyosin at low ionic strength is due to tropomyosin bound to the complex under these conditions. When tropomyosin is removed, the ATPase activity becomes as high as in the presence of Mg" and at the same time the actomyosin loses its EGTA sensitivity. The difference between the Mg" and Ca" activated ATPase of myofibrils at low ionic strength increases with further development. This might reflect a continuous rise of tropomyosin content of the myofibril. The EGTA sensitivity also increases significantly during the later stages of development; the EGTA sensitivity is realized by the joint action of troponin and tropomyosin. Thus, these regulatory proteins are still developing at a time when the enzymatic activities of the contractile proteins have reached the adult level. The authors also point out that the ATPase activity of red muscle is as high as it is for white muscle in the rat. This is in contrast to other work with rabbit where red muscle ATPase was much lower than that from white muscle. In rat, the resistance to inactivation of myofibrillar ATPase from red compared to white muscle was the only apparent difference between the 2 types.
The enzyme probably most studied during the developmental changes is lactic dehydrogenase. Five electrophoretic forms of lactic dehydrogenase are found in the muscle of animals; they represent two distinct types of enzyme (M and H) with 3 intermediate hybrids (Fine et al., 1963). When patterns do not follow a binomial distribution, more than one cell type is involved. The above researchers followed developmental changes in human skeletal muscle. The results were expressed as percent H subunits and they found 99% in muscle from 6 week old foetuses. Eighty-five percent in 3 month old, 60% in 7 month old and 12, 22 and 26% in 3 different adults.

Clausen and Hustrulid (1969) studied lactic dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in human foetuses ranging in age from 13-25 weeks gestation. They used locomotive (gastrocnemius, psoas major, sartorius) and posture (gluteus maximus, quadriceps femoris, soleus) muscles. During foetal development a linear steady increase in total lactic dehydrogenase activity as well as a linear decrease in the H/M subunit ratio of the isoenzymes was found. No significant changes were found in the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The changes found suggest a steady increased synthesis of lactic dehydrogenase M-subunits in human skeletal muscles during foetal development. The weekly changes in the total lactic dehydrogenase activity and in lactic dehydrogenase isoenzymes are lower in muscles involved in support than in those involved in periodic contractility. These findings, together with the literature available, are consistent with the morphological fact that foetal development of skeletal muscle mostly concerns the white muscle fibers and not the red muscle fibers.

Singh and Kanungo (1968) have also published results on lactic dehydrogenase isoenzymes in developing rat muscle.

Syromy and Gutmann (1967) studied the fast posterior latissimus dorsi and slow anterior latissimus dorsi in 20 day embryo, 1 day, 8 day and 30 day chicks. Their data suggested that differentiation may involve 2 different mechanisms, one essential for the fast muscle and reflecting a predominance of glycolytic processes, the other essential for slow muscle and reflecting a faster turnover of proteins.

CROSS-INNERVATION STUDIES

We have, up to this point, considered the histochemical, physiological and biochemical changes that are characteristic of the process of muscle fiber differentiation; however, we have left unanswered the question of what controls and regulates this process of differentiation. The beautiful experiments on cross-innervation shed some light on this question.

The initial experiment of Buller et al. (1960b) on nerve cross-union was considered earlier in the physiology section. You will recall that their postulate involved the actual passage of a substance down the axons. We shall consider now 4 additional manuscripts on cross-innervation that are pertinent to the aims of this review.

Dubowitz (1967) employed the slow soleus and fast flexor hallucis longus (FHL) or fast flexor digitorum longus (FDL) of newborn kittens and
rabbits and adult cats in his experiments. Cross-innervation produced
dramatic changes in the histochemical pattern of fast muscle with the
development of areas indistinguishable from normal soleus. The cross-
inervation FHL showed a striking appearance on enzyme histochemical
assessment. Areas of the muscle had a normal FHL pattern with a checker-
board of type I and II fibers, although with an apparent absence of inter-
mediate fibers. In addition, there were areas of muscle composed entirely
of type I fibers and indistinguishable from normal soleus. These latter
zones comprised either single or multiple bundles of fibers or at times only
part of a bundle. The converse change from the histochemical pattern of slow
soleus to that of fast muscle also occurred but was less consistent. When
fast muscle is reinnervated by soleus motorneurones, it produces a slower
twitch and a low maximum rate of tension development during isometric
tetanus--both are characteristic of normal slow muscle. Soleus innervated
by fast FHL motorneurones produces a faster twitch, but retains a low maxi-
imum rate of tension development during isometric tension. Thus, it falls
short of complete conversion and is an intermediate hybrid. Histochemically,
all the transformed cross-innervated muscles are hybrids. The incomplete
conversion may be due to some inevitable self-innervation, even when the
crossed nerves are well separated from each other. Dubowitz (1967) concluded
that neural influence determining the contractile properties of fast and
slow muscle has a profound controlling influence on the structure and meta-
bolic activity of the muscle fibers. It is interesting that he could not
demonstrate biochemically a significant change in the ATPase activities of
the fast and slow muscles following cross-innervation.

Romanul and Van Der Meulen (1967) studied cross union or reunion
of nerves to soleus and FDL or FHL in young and adult cats and rats. Cross-
inervated muscles reversed their speed of contraction and the enzymatic
characteristics of their fibers. Thus the high oxidative and low glycolytic
enzymatic profile of the soleus muscle fibers was changed to a low oxidative
and high glycolytic pattern of the normal FDL and FHL fibers. Converse
changes occurred in the FDL or FHL. Reinnervated muscles showed no change
in the speed of contraction or proportion of fibers of appropriate enzymatic
types. The results prove that the energy metabolism of the muscle fibers is
determined by the nerve supply, as interpreted by these authors. An addi-
tional observation made was that in normal muscle the fibers of different
histochemical types were scattered among each other. In cross-innervated and
reinnervated muscle, fibers of the same histochemical type were arranged in
small groups. Some groups of fibers of a single histochemical type and equal
size had a diameter which differed markedly from that of all other fibers
regardless of type. Romanul and Van Der Meulen (1967) thought this suggested
strongly that the motor units were histochemically homogeneous. Gross obser-
vation revealed that cross-innervated muscles were changed in color; i.e.,
soleus paler and FDL darker red.

Karpati and Engel (1967) studied the sequence of histochemical
changes from a mixed to uniform pattern in guinea pig soleus extrafusral muscle
fibers from 50th day gestation to 6 weeks postnatal and also the prevention
of that change by neonatal denervation. The soleus, in adult guinea pig, is
uniform with only type I fibers that are light with ATPase and phosphorylase
and dark with most other dehydrogenases. The soleus, in newborn guinea pig,
is mixed with 55-65% type II fibers. Type II fibers diminish up to 6 weeks
age when they are no longer present. In newborn kitten there are approximately
equal numbers of type I and type II fibers, and in adult cat there are occasional type II (≤3%) fibers. In newborn rat there is less differentiation and some myotubes are even present; about equal numbers of type I and type II fibers are apparent by 10 days postnatal and in adult there is greater than 90% type I. The loss of type II fibers in guinea pig soleus during maturation is prevented by neonatal sciatic denervation, but not by neonatal tenotomy. The mixed fiber population persists in denervated but the contralateral control muscle develops the adult pattern. The "loss" of type II fibers is delayed by nerve crush followed by reinnervation but does occur at a later time. The "loss" could be atrophy or transformation, but the authors favor transformation because: (1) absolute number of fibers increases, (2) diameter of type II fibers increases until they gradually disappear and (3) necrotic fibers are not seen. Two conclusions were reached. First, the maturing motor nerve has a decisive role in determining the normal histochemical profile of developing muscle cells. It is possible that this function of the lower motorneurone is dependent upon suprasegmental innervation and would be absent in deafferented lower motorneurones. Second, the authors state that the histochemical pattern of extrafusal muscle fibers in soleus of newborn animals is not well correlated with speed of contraction. They quote studies with cat (Buller et al., 1960a) that show at birth all muscles including soleus are slow twitch and within a few weeks certain (EDL and gastrocnemius) become fast twitch, whereas soleus remains slow twitch. The researchers (Karpati and Engel, 1967) think that the profound loss of type II fibers from soles of newborn rat and guinea pig, as they develop to maturity, should be accompanied by considerable physiologic slowing that has not actually been shown with cat. Therefore, they suggest that all type II fibers are not fast twitch physiologically, at least in developing muscle.

Further thoughts have been gleaned from cross-innervation experiments by Robbins, Karpati and Engel (1969) who studied the isometric contractile properties of guinea pig soleus muscles in vitro. Although solei are normally slow twitch muscles, solei cross-innervated by peroneal or tibial nerves (which normally innervate fast muscle) show speeding of isometric contractile properties: shortening of twitch time to peak, slight increase in maximum rate of isometric shortening and diminished build-up of tension during a low frequency tetanus. Normal guinea pig soleus are all histochemically type I, but after cross-innervation a variable percent of type II fibers appeared as defined by myofibrillar ATPase reaction. The physiologic data were consistent with the hypothesis that all fibers within the cross-innervated soleus are partially "speeded". The data were incompatible with the hypothesis that some fibers in X-innervated muscle are completely "speeded" becoming like normal fast fibers, the rest remaining completely slow. The correlations of percent of type II fiber per cross-sectional area with twitch time-to-peak or 5/sec tetanus:twitch ratio led to the interpretation that with the ATPase reaction, change in histochemical fiber type occurs when physiologic "speeding" exceeds a certain threshold. Figures are given to indicate the extent of type II fibers present. In all cross-innervated solei a variable number of typical type II extrafusal fibers of normal diameter and architecture appeared, either singly or in groups. The proportion of type II fibers, expressed as percent of total cross-sectional area of muscle at the midpoint, ranged from 5 to 37 percent with a mean of 18.3 percent; in self re-innervated and contralateral solei, no type II fibers were seen and in EDL the cross-sectional area for type II was about 88 percent.
The authors give calculations and reasoning that change in contractile properties of slow muscle, cross-innervated by a nerve normally innervating fast muscle, is present in most or all of its component fibers. Their results indicate that the percent of type II fibers acts only as an indicator of the degree of speeding of the whole fiber population. For instance, the postnatal developing rat soleus shows a progressive shortening of contraction time (i.e., "speeding") while the percent of type II fibers is actually decreasing. It is possible that while the mean contraction time is slowly moving in the direction of increased percent speeding, the histochemical "turnover point" is moving to a higher percent speeding at a still faster rate.

**SIGNIFICANCE TO MEAT RESEARCH**

We have examined in detail the process of fiber differentiation. We have done so because there is an important area of application to meat science; the fiber type composition of meat animals can markedly influence the post-mortem change and ultimate value for use as food (Cooper et al., 1969c). In order to understand and solve the problem, we must know how the fiber population develops and what factors influence the development of normal or abnormal fiber populations. Dubowitz (1963) stated in one of his early papers that lack of differentiation in muscle fibers in some diseases may represent a failure of maturation of the muscle and thereby a study of differentiation might yield valuable insight to understanding the disease. We thought likewise that abnormalities in the muscle of meat animals (such as Giant Fibers, Cassens et al., 1969b) might be better understood by having available a complete histochemical description of fiber differentiation.

Cassens et al. (1969a) have used the sudan black B technique to visualize red and white fibers in porcine longissimus muscle at the following developmental stages: 8-9 weeks gestation, 9-1/2-11 weeks gestation, 12-13 weeks gestation, 1 day postnatal, 13 day old, 180 day old and 24 month old. There was no differentiation of fiber types in any of the foetal stages or at 1 day of age. Much loose connective tissue was present in the foetal stages, the fibers were round rather than polygonal and the fibers were arranged in clusters that resembled primary bundles. The fibers from the foregoing stages were sudan black B positive and therefore taken as type I. The differentiation of fiber types was clear by the time the animal had reached 13 days of age and about 60 percent of the fibers were sudan black B negative or type II. Type I fibers composed only a small portion of the total fibers (about 15%) by the time the animal had reached 180 days of age and there appeared to be little change as the pig matured to 24 months of age. This work established the general aspects of fiber differentiation in pig longissimus muscle.

Cooper et al. (1969a) have completed an extensive histochemical study of fiber differentiation in pig muscle (Longissimus) by using DPNH-TR, amyl phosphorylase and ATPase techniques. In addition, they (Cooper et al., 1969b) have run a complementary study with biochemical techniques (phosphorylase, lactic dehydrogenase, glutamate-oxaloacetate transaminase and lactic dehydrogenase, glutamate-oxaloacetate transaminase and lactic dehydrogenase isoenzymes). The pigs were Poland China, Yorkshire and the reciprocal crosses between the ages of 1 day and about 6 months. Figures 2, 3, 4 and 5 show the process of differentiation as revealed with histochemistry. Fiber differentiation had not taken place at 1 day of age according to histochemical observations although the myosin-ATPase appeared to show some slight differentiation. At 1
week of age the type I fibers were distinguishable from the other fibers. Type II and intermediate fibers could be separately classified at 4 weeks of age. Percentage of a particular fiber type, calculated on a cross-sectional area basis, indicated that type II fibers increased over the period of 4 weeks to 6 months at the expense of intermediate fibers, whereas the type I fibers decreased only slightly. Number of fibers versus area of fibers gave a bimodal distribution for the 1 day old pig and a unimodal one for the 6 month old animal. Over the period studied there was a relationship between fiber size and age. Biochemical determinations of phosphorylase and lactic dehydrogenase activity increased over the age periods studied and agreed with the increase of histochemically classified type II fibers. The glutarate-oxaloacetate transaminase levels increased over the first few weeks and then fell as the animal grew older.

Morita et al., (1969) have employed the histochemical myoglobin technique to the same group of pigs described in the preceding paragraph. They found essentially a negative myoglobin reaction in longissimus muscle of 1 day old pigs. By 3 weeks the typical adult pattern was evident and the capillary distribution was clear and similar to that in adult muscle.

An additional observation, that might be quite significant, should be mentioned at this point. In all the figures shown (Figures 1, 2, 3, 4, 5), the arrangement of type I fibers is in the distinct clumps or groups--this is very unlike the checkerboard or scattered arrangement that is known for other mammalian muscle. This pattern is reminiscent of the small groups of one fiber type seen in cross-innervated or reinnervated muscle (Romanul and Van Der Meulen, 1967). The real importance of this observation in pig muscle is unknown but certainly merits further investigation.

Differentiation has also been studied in beef muscle (Waldman, 1967). He studied longissimus muscle from birth to about 1300 lbs animal weight and generally found a decrease in red fibers as the animal matured.

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Figure 1. Red and white fiber distribution in porcine longissimus muscle. The red fibers are grouped in clumps in contrast to the checkerboard arrangement that has been reported for other mammalian muscle. Secion reacted for DPNH-TR; A is 20X and B is 125X.
Figure 2. Sections (porcine longissimus muscle) reacted for DPNH-TR that show the differentiation of fiber types. A (1 day old; 496 X) shows a uniform strong reaction, but does reveal some large fibers and some small fibers. B (1 week old; 312 X) shows a differentiation of strongly and weakly reacting fibers, but the weakly reacting still display a granular pattern. C (2 week old; 312 X) shows a more distinct differentiation.
Figure 3. Sections (porcine longissimus muscle) that show the differentiation of fiber types. A (3 weeks old; 312 X) still shows that lightly staining fibers have a granular reaction pattern. B (4 weeks; 312 X) and C (5 weeks; 312 X) illustrate the time when white fibers begin to lose granularity and intermediate fibers can be separately classified. D Shows a section from 8 week old pig at 124 X.
Figure 4. Sections (porcine longissimus muscle) that show the differentiation of fiber types. A is from 16 week old pig at 124 X and B and C are from 20 and 24 week old pigs respectively at 50 X. From the period 4 weeks to 6 months, type II fibers increase at the expense of intermediate fibers whereas type I fibers decrease slightly.
Figure 5. Serial sections reacted for diphosphopyridine nucleotide tetrazolium reductase (A), amylophosphorylase (B) and ATPase (C). The greater selectivity of the ATPase is illustrated. All at 124 X.
BOB CASSENS: Paper In.

JIM KEMP: Thank you very much Bob and if we have time when we get through, we’ll let him show these. Now the next speaker will be using the other projector, so we will go ahead.

BOB CASSENS: Projector’s fixed so on with the slides.

JIM KEMP: Thank’s Bob for a very excellent paper despite the inconvenience. Our second speaker is from that "show me" state of Missouri. He showed me that he knows quite a bit about his subject when I read his recent paper that came from the Symposium on Body Composition in Animals and Man. Milt Bailey will now speak to us on "Changes in Proteins and Related Substances in Muscle During Growth and Development." Milt.

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