insulin(10,11,12,13). This conclusion is also supported by the notable similarity of the effects of tolbutamide to those of injected insulin(14) upon cardiac carbohydrate metabolism.

Summary. Tolbutamide, 40 mg/kg, was rapidly administered into the femoral vein of intact, anesthetized dogs, deprived of food overnight. One hour later the following were observed: a) a significant decrease in arterial concentration of glucose; b) a significant elevation in *percent* of blood glucose extracted by the myocardium without any significant change in *absolute* amount extracted or *total* amount utilized; c) no significant change in arterial concentration or myocardial extraction and utilization of pyruvate, lactate, oxygen, Na^+ or K^+; d) no significant deviation in cardiovascular dynamics including coronary blood flow and cardiac index.

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Changes in Phosphorus Metabolism of the Gastrocnemius Muscle in Aging White Rats.* (26630)

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In pursuit of evidence for the corollary to Sumner's classical statement(1) that "life is an orderly function of enzymes" *viz.* that senescence in structures exhibiting anatomical and functional decline with age should be reflected by corresponding alterations in activity of significant cell catalysts, a long-range study has been under way in this laboratory of the changes in enzymes related to metabolism of high-energy organophosphorus compounds in skeletal muscle of aging rats. Potter, *et al.*(2) reported a marked rise in adenosine triphosphatase (ATP-ase) activity in the skeletal muscle of the immediately postpartum rat. Moog(3) and Robinson(4) in developing chick muscle and De Villafranca(5) in developing rat muscle also found a corresponding rise in ATP-ase activity with development of fibrillar substance. As for the organophosphorus compounds (including the corresponding substrate ATP) there are conflicting reports for distribution of acid-soluble phosphorus compounds with age in human(6) and in rat skeletal muscle (7). A recent study by Rockstein, involving both the enzyme system(8) and the related adenine nucleotides(9), important in the energizing of muscular contraction related to flight in insects, revealed a marked decline in the Mg-activated ATP-ase and other enzymes and a concomitant accumulation of

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ATP, with decline and loss in the flight ability in males. This suggested a similar study on changes in enzyme activity and related adenine nucleotides in skeletal muscle of aging rats, particularly in a strain showing marked loss in muscular function in senile animals. This report summarizes the results of over 3 years of such a study.

Materials and methods. Tissue preparation. Male rats of the Sprague-Dawley strain (from the Hormone Assay Laboratories) were bred, reared and maintained in temperature-controlled animal quarters and fed *ad libitum* on a standardized commercial laboratory diet. Each animal was examined and weighed once weekly, beginning with the end of the third month. For each analysis an animal of known age was sacrificed by rapid separation of the brain from the spinal cord and rapid excision of the tissue under study. The gastrocnemius muscle was removed, *in toto*, cut transversely into 2 or 3 approximately equal sections according to size, then rinsed in ice-cold pH 7.4 Tris (Sigma) buffer solution, and dried briefly on paper towelling before being weighed. The sections were then plunged into liquid air and fast-frozen for 10 minutes before being transferred to capped polyethylene vials and stored in a freezer at −30°C until studied. For each enzyme (or substrate) determination, the frozen sample of muscle was cooled further in liquid air and then crushed into a powdery consistency in a previously cooled Elvehjem-Potter stainless steel crusher. The crushed tissue was then transferred quantitatively to an Elvehjem-Potter glass homogenizer cup containing 2 ml ice cold 7.4 Tris buffer and homogenized mechanically with a Teflon pestle for 2 minutes. The homogenate was transferred quantitatively (including rinses of pestle and homogenizer cup with ice-cold 7.4 Tris buffer) into a cold centrifuge tube made to a final volume of 5 ml of buffer and allowed to stand in an ice bath for 20 minutes at zero°C in order to extract water-soluble enzymes. After being centrifuged for 10 minutes at 2500 rpm (970 g) in a refrigerated centrifuge at zero° C the supernatant liquid was carefully decanted and stored in an ice bath for study of the Mg-activated (water-soluble) enzyme. Where the Ca-activated (actomyosin) enzyme activity was estimated, the centrifugate was resuspended in 7 ml ice-cold 0.6 *M* K Cl in pH 9.0 Tris buffer solution containing 0.002 *M* Versene (Na tetraversenate) as an "activator" of actomyosin ATP-ase. After elution by standing at zero°C for 20 minutes, the suspension was centrifuged at zero° C for 10 minutes at 2500 rpm and the (high ionic strength salt solution) extractable actomyosin solution decanted and stored cold. This brei was employed in determination of actomyosin (pH 9.0) ATP-ase activity.

Enzyme assay. To one ml of homogenate in 7.4 Tris buffer solution were added 0.1 ml each of ice-cold 0.03 *M* ATP and of 0.03 *M* $MgCl_2$, each prepared in the 7.4 Tris buffer solution. After addition of a drop of chloroform, the incubation tube was stoppered and mixed by inversion 3 times and transferred to a constant temperature water bath at 37°C. At the end of a 5-minute period required to reach temperature equilibrium with the water bath, a zero-time (control) 0.5 ml sample was removed from the freshly remixed incubation tube into a vial containing 0.2 ml of 20% trichloroacetic (TCA) and the contents thoroughly mixed. Fifteen minutes later, a second 0.5 ml aliquot was similarly removed from the incubation tube into a second vial of 20% TCA; this was the experimental sample. After 5 minutes to permit complete deproteinization, the mixture was filtered through a Whatman 42 (4.25 cm) filter disc folded into a one-inch glass funnel. Enzyme activity was determined in terms of differences between the inorganic phosphate content of 0.2 ml samples of deproteinized experimental and control filtrates. This was determined by the ferrous-sulfate-acid molybdate method of Rockstein and Herron(10). For each tissue preparation (3 per muscle), 5 such pairs of experimentals and controls were made for each pH level indicated. The gastrocnemius muscles from 10 animals were studied for each age group.

Results. Table I shows the changes with age of Mg-activated (pH 7.4) ATP-ase activity for both male Sprague Dawley and

TABLE I. Age Changes in Mg-ATP-ase Activity and Weight of Gastrocnemius Muscle of the Male White Rat.*

Strain	1 Median age (mo)	2 ATP-ase activity	3 Muscle wt (g)	4 Muscle wt/body wt ($\times 10^{-3}$)
Sprague-Dawley	9.5 (7-12)	.69 (± .13)	4.58	11.93
	16.5 (13-20)	.51 (± .18)	4.63	9.47
	26.0 (23-28)	.24 (± .07)	2.45	6.69
CFN	8.0 (5-12)	.79 (± .15)	4.10	8.92
	17.5 (14-18)	.58 (± .10)	4.89	9.34
	26.0 (24-33)	.37 (± .05)	4.55	7.91

* Values in parentheses, column 1, show actual ranges of ages for the various median age groups. Data in columns 2 and 3 represent median enzyme and gastrocnemius muscle mass values for 10 animals of each median age group. ATP-ase activity expressed in μg of P released in 15 min., per g of fresh muscle mass. Values in parentheses in column 2 are probable deviations.

CFN, specific pathogen-free, white rats. The latter were offspring (delivered by Caesarean section) from Carworth Farms females and nursed by Nelson, germ-free foster mothers. Median age groups indicated in column 1 are based on age-dependent, weight-growth curves (to be published) and the fact that the somewhat longer-lived CFN animals have a long middle age, during which the body weight remains unchanged, beginning with the 300 days after birth and through the 800th day, in contrast to the shorter-lived Sprague-Dawley males with the shorter middle age of steady weight occurring at 400 to 600 days after birth. Also shown are the total gastrocnemius muscle weights and muscle weight:body weight ratios. In the Sprague-Dawley strain the fall in enzyme activity was very marked, that of senile rats being only about 35% of that of young, growing animals, a fall of 65%. In the old CFN rats, on the other hand, enzyme activity falls about only 50%. Moreover, the total gastrocnemius muscle mass is seen to fall much more markedly than does the absolute body weight, *i.e.*, this ratio falls by 45%. This means that, in the physiologically senile Sprague-Dawley animal, the loss in Mg-activated ATP-ase is even greater on an absolute body weight basis (at least twice that which is expresed on a total muscle weight basis). In the CFN strain, on the other hand, there is virtually no change in muscle to body weight ratios from young to old age and, therefore, the enzyme activity available on a body weight basis to the senile CFN males is considerably more than twice that found in the shorter-lived strain with a shorter constant weight middle age and a longer period of physiological senility.

It is of considerable interest (*See Discussion*) that no changes were found for either strain in the actomyosin (pH 9.0) ATP-ase activity nor in the adenosine triphosphate (ATP) and adenosine monophosphate (AMP) content of the gastrocnemius muscle, with advancing age.

Discussion. Two major considerations are implicit in these data. The first concerns the completely different picture as regards changes in the Mg-activated (pH 7.4) ATP-ase and the actomyosin (pH 9.0) enzyme, with advancing age. Although the actomyosin dephosphorylating system must be considered more directly concerned with the process of contraction in skeletal muscle, it it remarkable that its specific activity remains unchanged in a strain showing pronounced failure in motor function in very old animals. On the other hand, the activity of the Mg-activated enzyme shows a tremendous fall with advancing age in this same strain. This is especially significant inasmuch as De Villafranca (5, *op. cit.*) has also demonstrated conclusively that the Mg-activated ATP-ase shows a pronounced rise in activity during development and maturation of skeletal muscle in the post-partum rat up to about 30 days. De Villafranca suggested that the Mg enzyme may be involved here in protein synthesis during formation of the myofibrillae. However, the Mg-activated en-

zyme system has been studied extensively at the Wenner-Grens Institute by Low *et al.* and other colleagues of Lindberg(11). They suggest that the skeletal muscle Mg-activated ATP-ase serves as a transphosphorylating catalyst between ATP and reduced diaphorase flavin, and is therefore an important enzyme in the complex of accessory reactions involved in the oxidative aspects of phosphorylation. Thus, both maturation of the contractile mechanism, in the case of the *developing* rat, as well as in the gradual failure of motor function in the *senescent* rat, indicate a direct or indirect role of the effectiveness of the phosphorylating mechanism in the myofibrillae at different ages. However, in the case of the rat skeletal muscle, our findings that there is no change in ATP or ADP content on a unit muscle-weight basis despite the marked fall in activity of the Mg-activated ATP-ase, suggest that alternative pathways may be operating in oxidative phosphorylation in aging rat skeletal muscle. This is also evident from the above-reported strain differences. It is interesting to note, for example, that gross manifestations of functional decline are apparent in the strain which shows a muscular dystrophy-like syndrome of the hind limbs; concomitantly, the specific activity of the Mg-activated enzyme in the gastrocnemius muscle falls very markedly and, on an absolute body weight basis, even more drastically. In the CFN strain, on the other hand, with a greater life span, a longer period of middle age, with constant maximum age and only a moderate decline in motor function (with no outward signs of the above-mentioned muscular dystrophy-like syndrome) in extreme old age, this enzyme system, on a *unit muscle mass basis* shows a smaller percentage loss in activity with extreme old age. With the muscle mass to body weight ratio remaining practically unchanged in CFN animals, final enzyme activity in the gastrocnemius muscle of this strain *on an absolute body weight basis* is at least twice as great as that of the Sprague-Dawley animals. Thus, whatever the exact role which this enzyme system may play in the effective utilization of the skeletal muscle components of aging mammals from birth to senility, we have in the Mg-activated ATP-ase system a firm, reproducible biochemical criterion for senescence of skeletal muscle function.

Summary. Changes in the adenine nucleotide dephosphorylating system in the gastrocnemius muscle of aging male rats have been studied. The Mg-activated ATP-ase activity showed a pronounced fall from young to very old (senile) age in both the Sprague-Dawley and the CFN strains. However, in the former strain, old animals, typically exhibiting markedly greater losses in muscle mass and concomitant muscular dystrophy, also showed a greater decline in this enzyme on a unit muscle mass and even more so on an absolute body mass basis, than did the old CFN rats. In both strains, however, actomyosin (pH 9.0) ATP-ase as well as ATP content showed no significant changes from young to very old age. The role of the Mg-activated ATP-ase enzyme in energizing of skeletal muscle contraction and its possible significance in the physiology of senescence are discussed.

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