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Enzyme Changes within Muscle Fibers in Genetic and Nutritional Muscular Dystrophy* (33327)

LOUIS L. TUREEN AND ROBERT SIMONS

Max and Anna Tureen Neurology Research Laboratory, Department of Neurology and Psychiatry, Saint Louis University School of Medicine 63104

Recent publications (1, 2) emphasize the relationship between the functional activity of muscle fibers, their innervation and their enzyme systems. Fast acting "white" fibers display strong activity for glycolytic enzymes, e.g., phosphorylase (Phlase) and are poor in the mitochondrial enzymes such as succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH). The reverse is true for cardiac muscle or other red fibers (3) which are more slow acting and require abundant oxidative enzymes. The pattern of reciprocal relation between levels of oxidative and glycolytic enzymes in functionally different muscle fibers is widely observed in rodents and birds, as well as in man (4). In the genetic dystrophic chick, there is a failure in the early posthatching period of maturation of the red, undifferentiated embryonic fibers to the white mature fiber which is the more abundant and characteristic fiber of the mature chicken pectoralis muscles. There is a corresponding failure or delay in the development of the enzyme systems usually found in mature pectoral muscles (5). Since the pectoralis muscle of the chicken is affected early and most severely in both the genetic and nutritional types of muscular dystrophy, the probability is strongly suggested that the metabolic activity of the white muscle fiber may be related to this early susceptibility.

The present experiments were designed to compare the histological changes in the dystrophic muscle fibers with concomitant levels of enzyme activity in them. One aim was to investigate whether the dystrophic necrosis results from a specific enzymatic disorder within the muscle fiber, or if the changes observed in both genetic and nutritional chicken muscular dystrophy represent nonspecific, generalized muscle responses to the stress of differing metabolic insults.

Methods and Materials. One-day-old New Hampshire chicks of the NH_2 strain (normal) and the NH_3 strain (genetic dystrophic) were used. The control group of normal chicks was placed on a standard commercial[1] diet as a starter feed for 30 days, and then transferred to a well balanced stock diet for optimal growth[2] for the remainder of the experiment. One group of the genetic dystrophic strain was similarly fed. The other two groups, one of the NH_2 strain, the other of the NH_3 strain, were fed a purified diet deficient in vitamin E and sulfuramino acid, used previously (6) to induce nutritional myopa-

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[1] Ralston Purina Co., Starteena brand.

[2] Ralston Purina Co., Growean brand.

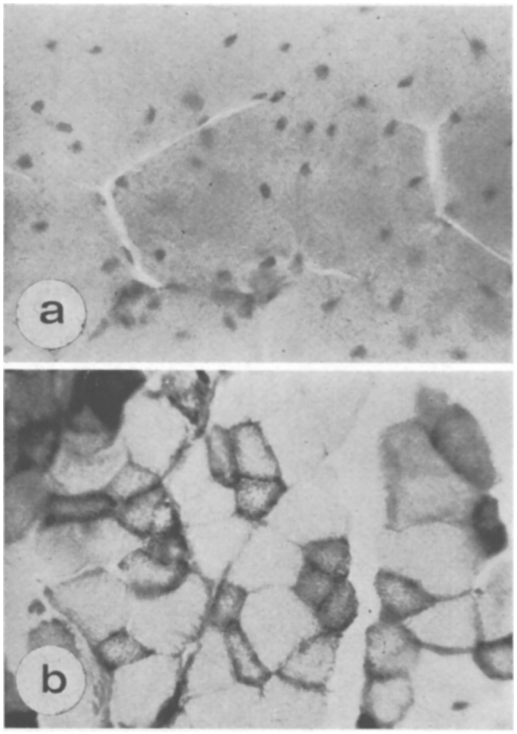

FIG. 1a. Superficial pectoral muscle: normal chick, H and E stain. Note variation in fiber cross section size, and internally placed nuclei. ×400. (b). Gastrocnemius muscle: normal chick; prepared to show DPNH distribution. The smaller "red" fibers show deposits of the enzyme activity which is scarce in the larger white fibers. ×100.

thy in chickens. Each group contained 6–10 chicks. All chicks were tested for their ability to right themselves; inability to do so in 7 out of 10 tests indicated a moderately severe degree of paralysis.

Biopsies were obtained (under Nembutal anesthesia) of deep and superficial pectoral muscles and of gastrocnemius muscle at various stages of paralysis, and from control birds. The tissue preparations were promptly frozen in isopentane in a liquid nitrogen bath at —160°. Frozen tissues were subsequently sectioned at 10 μ in a cryostat at —20 to —30° and stained on cover slips with H and E and Gomori trichrome stain for conventional histological preparations, and by appropriate methods to demonstrate some oxidative enzymes as well as phosphorylases, e.g., diphosphopyridine nucleotide diaphorase (DPND) (7, 8), LDH, SDH (9) cytochrome oxidase (10), α-glycerophosphate dehydrogenase (αGPDH) and amylphosphorylase (11).

Results. Normal strain chicks (NH_2) on the commercial diet developed normally. The three other groups, NH_2 amd NH_3 on the purified diet, and NH_3 on the commercial diet, all developed pectoral muscle paralysis by 12 weeks. The degree of disability seemed more severe in those chicks on the purified deficient diets.

It is pertinent to emphasize that the normal chick pectoral muscle (Fig. 1a) is characterized by irregularity of size of the muscle fibers, which contain nuclei within the sarcoplasm as well as in the subsarcolemmal spaces. Cross sections of these fibers appear polygonal. Smaller fibers, staining more eosinophilic than the larger, paler staining

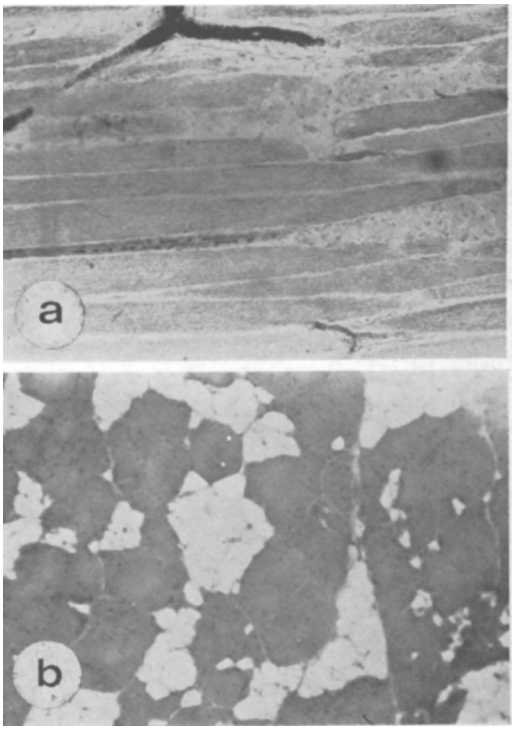

FIG. 2a. Pectoral muscle with nutritional dystrophy: prepared for DPNH activity. Longitudinal preparation to demonstrate focal alterations within single muscle fibers and the loss of enzyme activity within the damaged portions of the fiber. ×100. (b). Pectoral muscle with nutritional dystrophy: H and E stain; cross section shows rounded contours, more centrally placed nuclei than in the normal. Note extensive interstitial fat infiltration. ×100.

fibers, when prepared by methods to demonstrate various enzyme activities, exhibit the enzyme characteristics of red fibers, with more abundant activity of mitrochondrial enzymes, e.g., SDH and LDH as well as DPNH (Fig. 1b). Phosphorylase reactions in the larger fibers are more intense than in these smaller fibers. A number of intermediate fibers are present. Normal gastrocnemius muscle exhibits fibers of uniform size, with abundant peripherally placed subsarcolemmal nuclei. Central nuclei are not as abundantly found as they are in the pectoral muscle. Approximately one third of the fibers have staining characteristics and enzyme reactions of red fibers. Of the remainder, white and intermediate fibers are of approximately equal numbers.

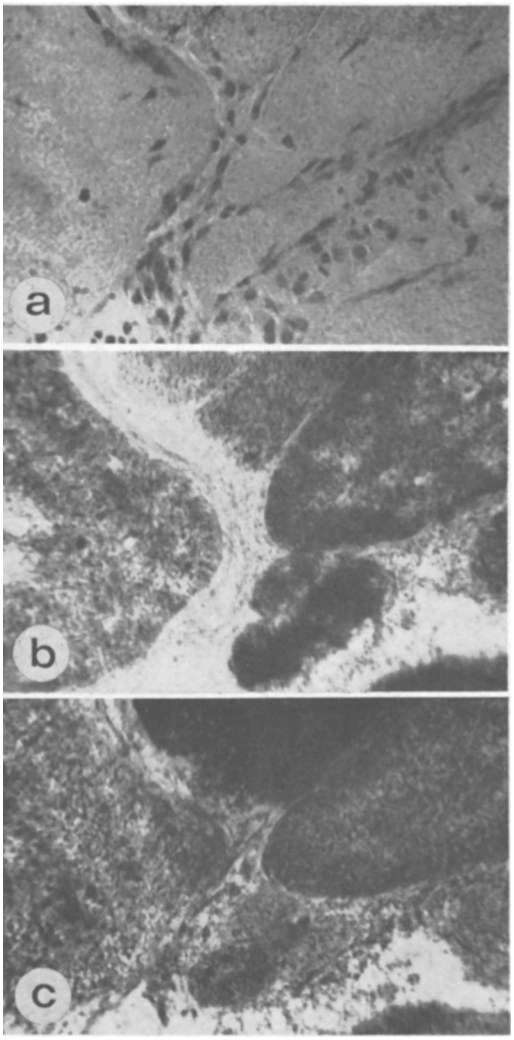

FIG. 3. Superficial pectoral muscle from chicken with genetic muscular dystrophy: serial sections to compare H and E appearance (a) with the α GPDH activity (b) and LDH activity (c) in large white fibers. Note that the glycolytic and oxidative enzyme activities are intense in the enlarged white fibers. ×400.

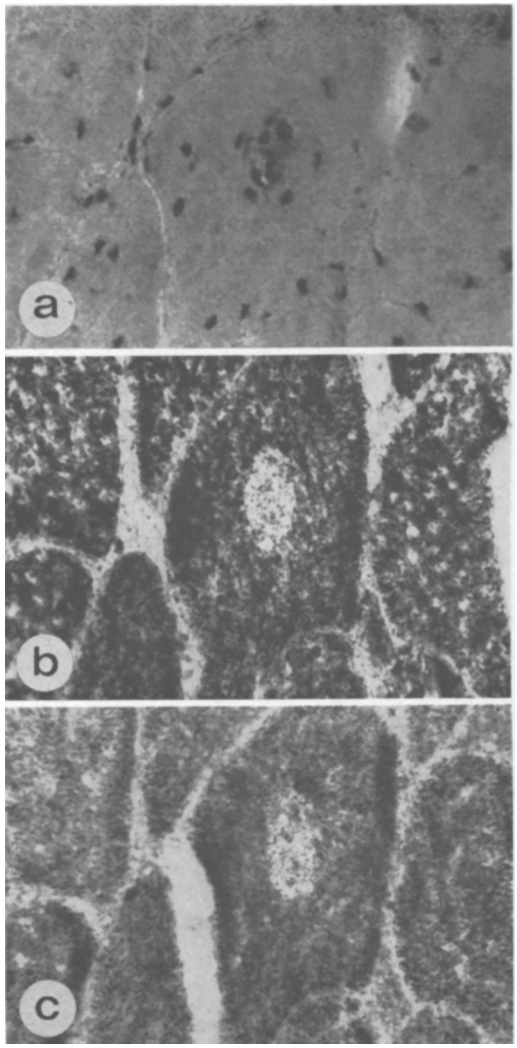

FIG. 4. Serial sections of genetic dystrophic pectoral muscle fibers: (a) H & E stain, showing accumulation of centrally placed nuclei; (b) αGPDH; and (c) LDH enzyme reactions. Note absence of enzyme activity in the center of the fiber with relatively intense reaction in periphery of the fiber for both glycolytic and oxidative enzymes. ×400.

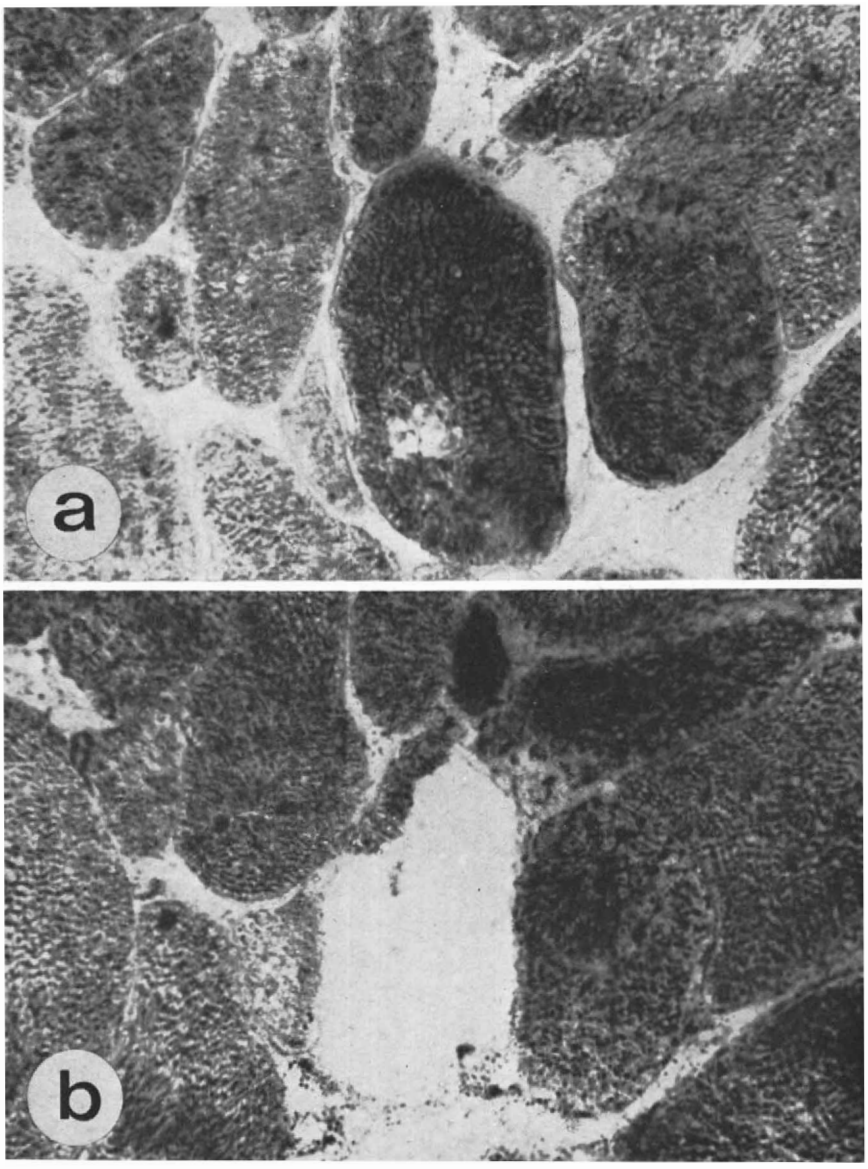

FIG. 5. Genetic dystrophic pectoral muscle fibers: (a) αGPDH preparation shows beginning loss of enzyme, (b) LDH preparation. Complete loss of enzyme of one fiber, which is seen almost only in outline; intense reaction in others. ×400.

The dystrophic process within the pectoral muscles of affected chickens is alike in each of the three groups which developed lesions, with the exception that in the nutritional dystrophy, necrosis is more intense than in the genetic disorder; also an intense interstitial reaction, such as is encountered in myositis is observed. The necrotic process is a focal one, affecting individual fibers, or portions of individual fibers. Fibers tend to become hypertrophied, in whole or in part; they may split in whole or in part, whether they become hypertrophied or not. A single affected fiber may be compressed between normal appearing fibers either of usual size or considerably hyperthrophied (Fig. 2a). Some of the hypertrophied fibers exhibit more than normal numbers of centrally placed nuclei,

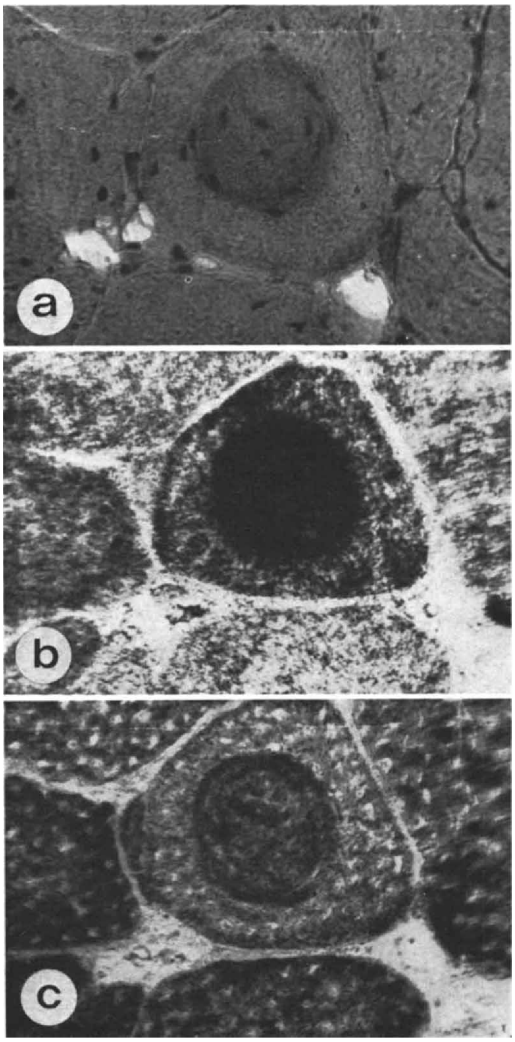

FIG. 6. Genetic dystrophic pectoral muscle fibers: (a) H & E stain; central core with basophilic appearance; (b) LDH preparation; (c) αGPDH preparation. Note intense enzyme reactions in central core, normal reactions in the periphery of one fiber.

which may occur singly or in clusters. In cross section, there is pronounced variability in the size of the fibers, which tend to become rounded in outline (Fig. 2b). It is of interest that some of the larger fibers, which with H & E appear to stain normally, will show intense reactions for αGPDH as well as for LDH, quite unlike the reactions usually observed in the large fibers of normal chick pectoral muscle (Fig. 3a,b,c). In other enlarged fibers, where clusters of nuclei occur within the fiber, reactions for the various enzymes examined are uniformly diminished. In Figures 4a,b,c, is demonstrated a fiber in cross section in which the central core of the fiber fails to react for such oxidative enzymes as LDH or SDH while the surrounding periphery of the fiber does react for these enzymes. Some fibers demonstrate intense reaction for αGPDH with loss of enzyme in the central core, while the adjacent serial section shows no response to methods for SDH or LDH (Fig. 5a,b). An occasional fiber exhibits in the H and E stain a central core of basophilic appearance, surrounded by normal looking cytoplasm. In the central core of such a fiber, an intense staining reaction appears (Fig. 6a) while the remainder of the fiber reacts normally for αGPDH and other phosphorylase reactions (Fig. 6b,c). In later stages of the degeneration, coagulation necrosis may affect an entire fiber. Also, one segment of the fiber may be wider than the rest, and empty sarcolemmal tubes compressed by adjacent fibers and lined by rows of sarcolemmal nuclei, may be encountered. These degenerating fibers are devoid of all histochemical indications of enzyme activity (Fig. 7a). Groups of adjacent fibers may be replaced by fat cells (Fig. 2b) and the extent of muscle loss seems to be important in the deposition of fat, which is the same in distribution and amount in both the genetic and nutritional disorders.

Evidence of unequivocal regeneration of fibers has not been seen in these preparations. Unquestionably the process is a continuous one of progressive necrosis and loss of muscle substance and enzymes. The dystrophic process in the gastrocnemius muscle occurs later, and more slowly; otherwise the process is like that in the pectoral muscles.

Discussion. There is a constant increase in the oxidative enzyme level in the white fibers of the pectoral muscle of the dystrophic chicken. Normally these enzymes are not abundant in these muscle fibers. It is now known that denervating a muscle fiber, or changing its function by altering its innervation, alters its enzyme systems (2). Thus it is not surprising that in the development of a

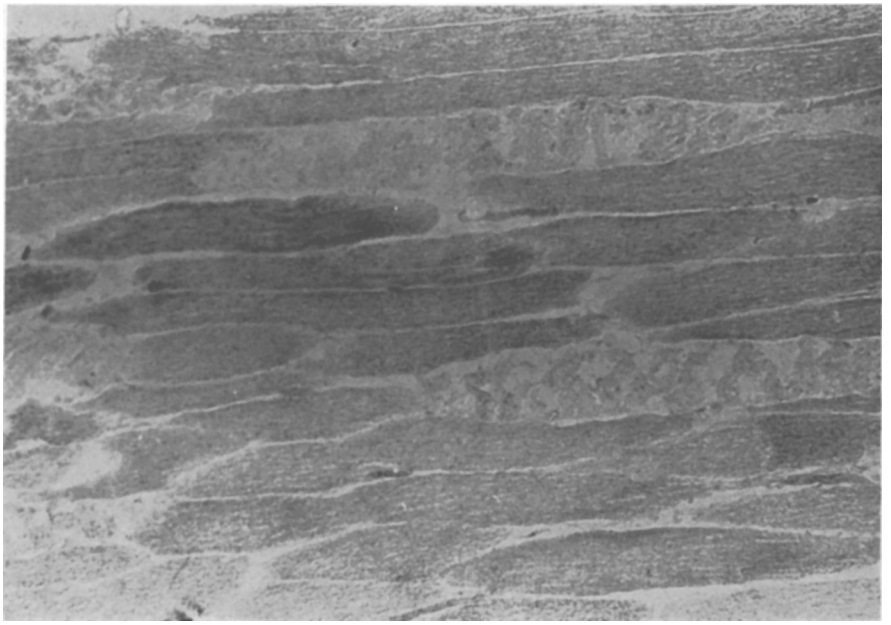

FIG. 7. Nutritional dystrophic pectoral muscle: preparation for DPNH demonstrating variation of size of portions of muscle fibers and loss of enzyme activity in damaged fibers.

pathological process as in a myopathy that enzyme systems within fibers change; in fact this finding parallels the observation that there is a failure of maturation of pectoral muscle fibers in the genetic dystrophic chick (5). The changes in our preparations were observed even when the fibers appeared histologically normal, or were simply hypertrophied. Similar changes occur in both the nutritionally induced and in the genetic dystrophic processes. This suggests that similar metabolic disturbances occur in both conditions.

When necrosis appears, enzymes escape from the fibers. Whether they enter the blood stream to elevate the serum enzyme levels cannot be stated now. It seems probable that this occurs. The conflicting reports (12, 13) of enzyme assays in muscle homogenates may well depend on the state of the muscle at time of the biopsy. In our laboratory, preliminary assays show an increase in serum LDH levels with a concomitant decrease in muscle homogenate LDH levels. Possibly, if biopsies were made at an earlier stage, higher homogeneate LDH levels would be obtained. These are matters for further examination.

Of particular interest in the peculiar vulnerability of chicken pectoral muscle to necrosis in both the genetic and nutritional disorders. White fibers are affected preferentially (14), with an early excessive content of oxidative enzymes. In this connection, Pappenheimer's observation that denervated muscles (which lose their enzyme reactions) are not affected by nutritional myopathy, indeed suggests a role for the enzymatic changes leading to the necrosis. Further investigation of these factors, particularly by electron microscopy combined with histochemical methods should be useful in pursuing this question.

Summary and Conclusions. Biopsy preparations of pectoral and gastrocnemius muscles from normal and genetic dystrophic chicks, and from chickens developing myopathy following diets deficient in vitamin E and sulfuramino acids, were examined histologically; serial sections were prepared to demonstrate activity of several glycolytic and oxidative enzymes. Histological and histochemical characteristics of genetic and nutritional myopathies are similar in pectoral and gastrocnemius muscles, but more intense in the former. Coagulation necrosis attacks individual fibers or portions of fibers in the earlier

stages of the disease. Before other histological alterations appear, white fibers enlarge and manifest the oxidative enzyme characteristics of red muscle fibers. Apparently, muscle fibers early in the myopathic process contain more enzymes, both oxidative and glycolytic, than do the normal muscle fiber. With the development within a muscle fiber of coagulation necrosis, reactions for both oxidative and glycolytic enzymes are diminished or completely absent. The early and extensive vulnerability of the pectoral muscle of chickens for dystrophic change is an index of its high white fiber content. With reference to enzyme activity in the muscle fibers undergoing myopathic changes, apparently two processes occur during the process: at first some enzymes are increased in amount; then when necrosis occurs, various enzymes examined in this study are lost from the damaged fibers.

It is not yet possible to state that a specific enzyme disorder is responsible for the dystrophic process. The similarity of lesions in the genetic and nutritional dystrophic disorder in the chickens suggests that a similar type of disturbance in metabolism is present in both conditions.

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Cytochrome C Reductase Activity of Meningopneumonitis Organisms at Different Stages of Development* (33328)

AKIRA TAMURA[1] AND G. P. MANIRE

Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

The psittacosis organisms undergo a developmental cycle consisting of a resistant mature infectious form (elementary body or EB) and a fragile intermediate reproductive form (reticulate body or RB) (1). Studies on metabolic activities in these organisms have usually been done with mixed population of EB and RB. In 1957 Allen and Bovarnick (2) reported the existence of NADH-cytochrome C reductase activity in a mixed population of meningopneumonitis organisms (MP) purified from allantoic fluid of infected chick embryos by the use of trypsin and differential centrifugation. More recently Allen used potassium tartrate density gradient centrifugation to obtain partial separation of EB and RB, with an upper band containing 80–90% of RB and no enzymatic activity, whereas the sediment contained 90% EB and almost all the enzymatic activity and infectivity of the original suspension (3).

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[1] Present address: Institute for Virus Research, Kyoto University, Kyoto, Japan.