8. Jerne, J., Nordin, A. A., Henry, C., Fuji, H., and Koros, A., Personal information. Information Exchange Group No. 5. Immunopathology. Scientific Memo No. 46, 1965.

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Phenotypic Expression in Chickens Heterozygous for Hereditary Muscular Dystrophy* (32772)

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Hereditary muscular dystrophy in the chicken is transmitted as an autosomal recessive trait (1). Characteristic elevations in plasma creatine phosphokinase (CPK) activity resembling those in human Duchenne muscular dystrophy have previously been reported for the dystrophic chicken (2). In addition, myopathic chickens show other plasma enzymatic alterations found in the human disease, including increased activities of glutamic-oxalacetic transaminase (GOT) and aldolase (3). Recent reports have indicated that serum CPK elevations are also present in the majority of female "carriers" of human dystrophy, despite generally asymptomatic muscular systems (4,5). To determine if similar alterations were present in chickens heterozygous for muscular dystrophy, plasma CPK and GOT activities were measured in "carriers" of this myopathy.

Materials and Methods. Fifteen chickens of the New Hampshire strain were bred for the heterozygous state by fertilizing dystrophic hens with normal rooster semen (backcross technique). The birds were raised on a balanced commercial diet along with genetically normal and genetically dystrophic groups with experimental conditions as previously described (2). Between the ages of 8–20 weeks, the birds were tested serially with the "exhaustion righting test," (2,3) and scores were recorded on the basis of total successes up to ten trials. Thus a chicken which when placed on its back, rose from the supine position five times but failed on the sixth trial, was assigned a score of five. Blood samples were collected (from unexercised birds) and heparinized with the post centrifugation plasma frozen for various periods of time before assay, always well within the allotted limits (6). Serial blood specimens were taken from the heterozygotes to trace the evolution of possible CPK alterations and to achieve a representative mean over the duration of the experiment. Plasma GOT was followed serially only in the first four chicks. Heterozygote chickens were sacrificed at approximately 20 weeks of age, at which time the pectoral muscles were inspected for gross dystrophic changes (1). Specimens of pectoral muscle were taken for histological evaluation. Duplicate assays for CPK (7) and GOT (8) were performed according to the routine colorimetric procedures of the Sigma Chemical Company with sulfhydryl activation used in the CPK procedure. Revised CPK units are expressed as the phosphorylation of 1 mμM of creatine per min at 25°C being equal to 1 unit (in extrapolation, this "revised" unit is lower by a factor of 16 as compared with the previously defined unit). The GOT determinations are expressed in Sigma–Frankel units (8). All results were evaluated statistically with the paired t test.

Results. The normal chickens showed no limitation in righting themselves when placed on their backs always achieving at least a score of 10 and frequently 15–25 as previously noted by Asmundson (9). Dystrophic chicks showed impaired "righting ability," beginning at the fourth week and progressing to complete loss by the eighth to the twelfth week. The heterozygote chicks were intermediate with every chick showing a less

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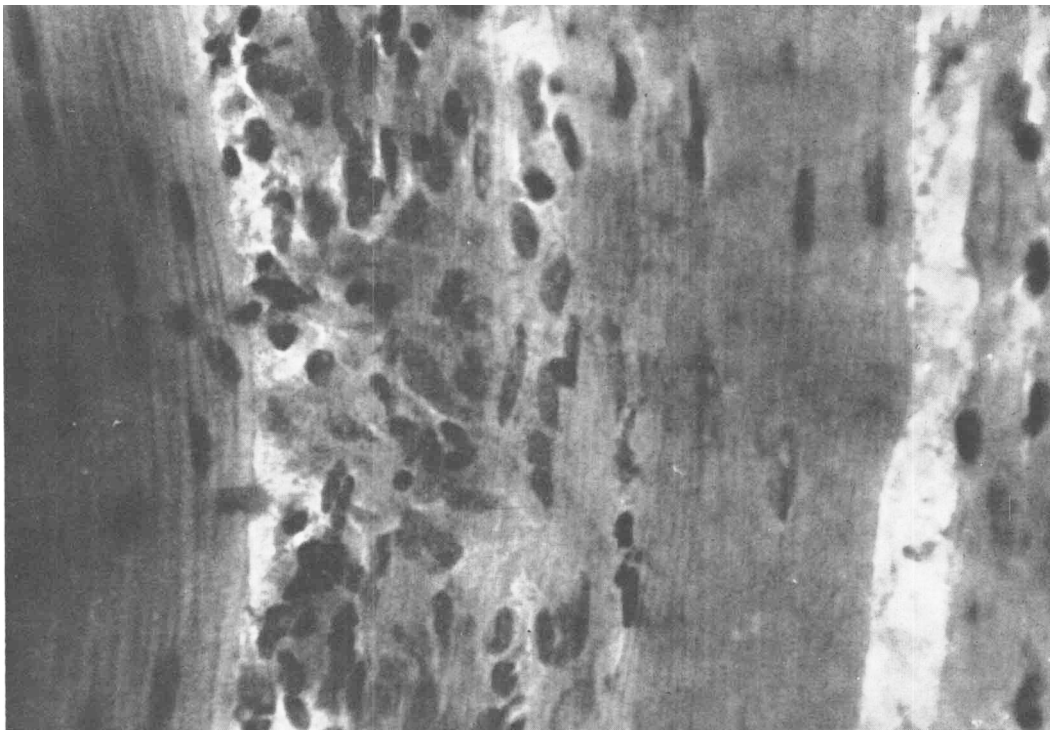

FIG. 1. Pectoralis muscle, heterozygous male chicken, age 16 weeks. Magnification 450 × 6. Longitudinal section. Demonstration of single fiber necrosis and infiltration.

than normal score at some time in the experiment. The average for all tests of heterozygote "righting ability" was 6.6, and a comparison with age shows no definite pattern of alteration (Table I).

Gross examination of pectoral muscles at sacrifice confirmed previous findings (1,10) for the normal and dystrophic chickens, the latter chiefly showing hypertrophy, interfascicular white fibrous striations, firmness, and resiliency to pressure. The heterozygotes showed variation in gross pectoral appearance with definite foci of white striations in two birds, questionable striations in two, and perfectly normal appearing muscle in the remaining eleven birds. One of this group showed mild pectoral atrophy but there was no evidence of hypertrophy in any animal by gross visual examination. Histological evaluation of the heterozygous pectoral muscle (Fig. 1) revealed mild dystrophic changes as follows: individual muscle fibers in the midst of a fascicle of normal fibers exhibited coagulation necrosis through all or part of the fiber. In other areas, examples of fiber splitting in parts of single fibers could be demonstrated. Phagocytic invasion of necrotic fibers were commonly encountered. Perhaps a greater variation in size of the fibers as seen in cross section was encountered when comparison is made with normal fibers (Fig. 2). The changes in the heterozygous muscle were qualitatively like those found during the necrosis stages in dystrophic muscle. The real difference was in the extent of muscle fiber involvement (Fig. 3). Actually one may say that the heterozygous

TABLE I. Weekly Tabulation.

Age	Righting test	CPK
8	7.5	104
10	6.6	122
12	5.7	158
14	4.2	120
16	6.1	298
18	5.6	332
20	6.5	354

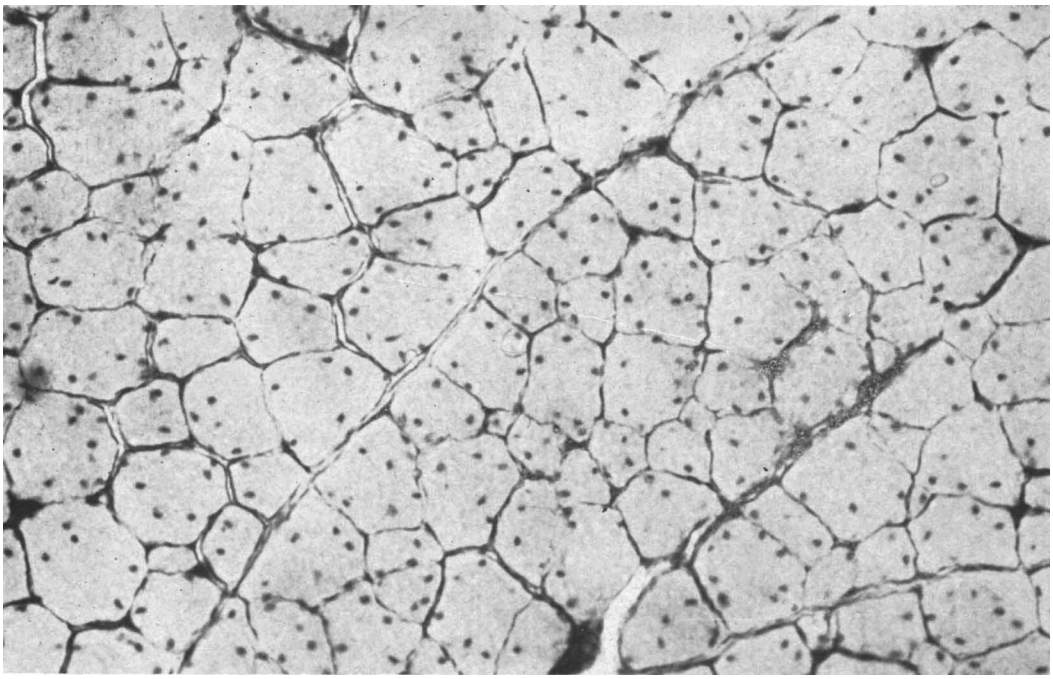

FIG. 2. Pectoralis muscle, normal male chicken, age 16 weeks. Demonstrating the relative uniformity of muscle cross section, with some variations in size, and with normally placed central sacrolemmal nuclei. Magnification 100 × 6.

muscle was intermediate between normal and dystrophic.

Data describing plasma CPK activities are presented in Table II. Mean CPK activities for the normal and dystrophic groups confirmed the previously reported values (2) and magnitude of enzyme elevation in the dystrophic chickens. (As expected with the new, revised assay procedure, both means were proportionally lower by a factor of 16 when compared with the previous findings.) The heterozygous groups show a significant ($p < .001$) increase in creatine phosphokinase with a mean of 165. Individual values indicate that with the exception of only three (20%) heterozygotes, all birds exhibit values above the upper limit of normal: 56 ± 52, mean ± 2 standard deviations (normal range). Heterozygote CPK arranged according to age (Table I) reveals that during this experiment, the elevated plasma activities for the group showed a sharp augmentation at ages 16–20 weeks. In examining individual heterozygotes, no definite correlation was noted between the level of CPK elevation and the degree of righting ability impairment; and no sex differences were noted.

TABLE II. Group Data. (Sigma units per ml)

Group	Mean CPK ± SD (No.)	Mean GOT ± SD (No.)
1. Normal	56 ± 26 (25)	161 ± 78 (16)
2. Heterozygote	167 ± 82 (15)[b]	288 ± 110 (12)[a]
3. Dystrophy	1012 ±788 (25)[b]	408 ± 213 (25)[b]

[a] $p < .005$.
[b] $p < .001$.

Mean SGOT activity for the dystrophic group was significantly elevated to 408 as compared with the normal mean of 161 (161 ± 156, normal range). The heterozygote group also showed a significant elevation in mean GOT activity to 288 ($p < .005$) with a great individual variation. Serial study of the first four chicks revealed a possible decline in the level of elevation between weeks 14–20 as compared with 8–14; but this sample size is too small to permit meaningful conclusions to be drawn.

Discussion. The findings presented herein

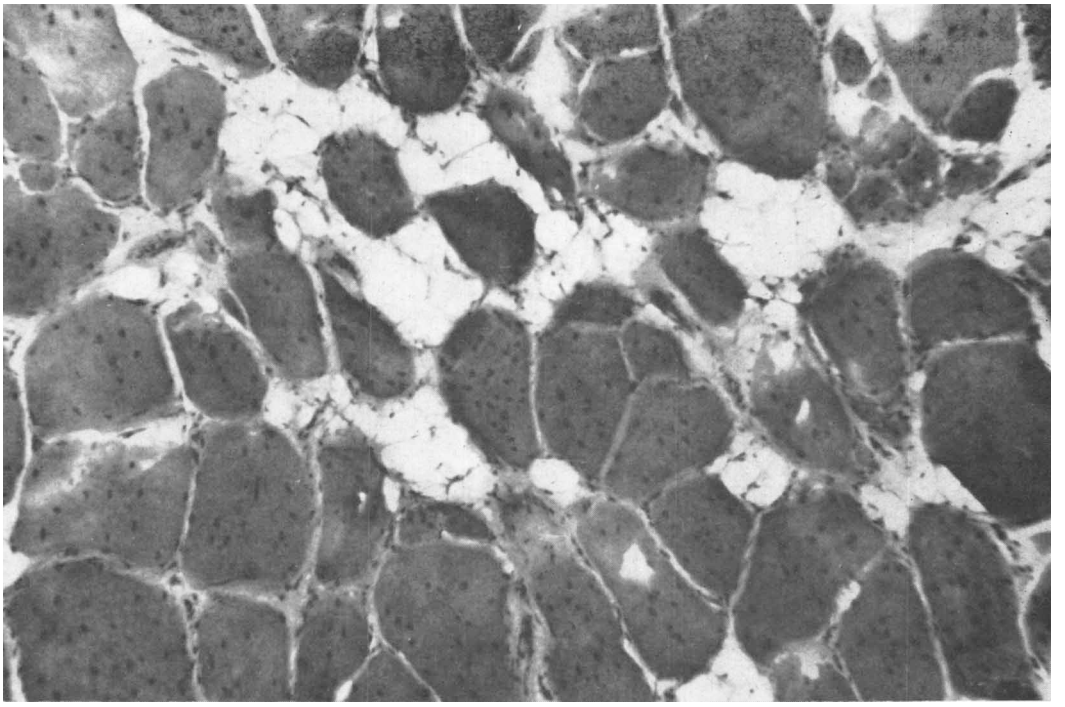

FIG. 3. Pectoralis muscle, genetic dystrophic male chicken, age 16 weeks. Intracellular fat infiltration is pronounced. Fibers are more variable in size and contain more centrally placed sarcolemmal nuclei than in the normal. Magnification 100 × 6.

suggest that chickens heterozygous for muscular dystrophy show intermediate, mild manifestations of the myopathy, as determined by (i) objective functional ability of the pectoral muscles, (ii) plasma CPK and GOT activity, and (iii) pectoral muscle histopathology. Asmundson and Julian (1), utilizing heterozygous chickens have also observed intermediate histological alterations, and in addition found an increase in pectoral muscle weight. Concerning plasma enzymes, the same workers (9) have reported that "CPK activity in 19 week old birds cannot be used to identify the carrier". More recently, however, Holloday *et al.* (11) have described elevated plasma creatine phosphokinase in a large percentage of older heterozygote chickens (1 year old). Employing the same exhaustion test used in this experiment, Chung (3) was unable to differentiate the heterozygote from the normal chicken. Thus our findings would appear to conflict with others in regards to clinical impairment of pectoral muscle function and elevation of plasma CPK at younger ages. Perhaps our laboratory's experimental conditions might explain the apparent discrepancies, especially since these birds are afforded little freedom of movement as previously described (2). Acceleration of the disease process by our experimental conditions is further suggested by comparing our findings of plasma CPK alterations (2) in dystrophic chicks (occurring early) with those of Holloday (occurring later) (11). In this connection, Asmundson (11) has found that frequent exercise delays the onset of dystrophy; and Homberger (12) has referred to the helpfulness of accelerating the myopathic disease process in studying animal dystrophy.

In the field of inherited animal muscular dystrophy, studies have dealt with the chicken, the mouse, and the Syrian hamster. In all three the disease is of autosomal recessive origin and resembles human dystrophy with elevations of plasma CPK and other enzymes (13–15). However, both 10–11 week old mice heterozygotes (13) and 18–31 week old ham-

sters (12) heterozygous for dystrophy show normal plasma CPK levels and no subjective muscle impairment of histological alterations. The heterozygous chicken as reported in this experiment differs from the other animals by manifesting alterations in all three parameters.

Female carriers of the sex-linked recessive human muscular dystrophy show elevated plasma creatine phosphokinase (4,5) but no consistent changes in other enzymes such as GOT and aldolase (4). Definite histopathological changes resembling dystrophy were found by Pearce (18) in quadriceps biopsy specimens from carriers of the muscular dystrophy trait, all of whom showed elevated plasma CPK. Although these individuals are generally asymptomatic, a small proportion do show clinical evidence of myopathy (16, 17). Despite the differences in inheritance pattern, it would appear that the carrier states of human and chicken dystrophy show basic similarities as regards the above discussed parameters.

The finding of positive biochemical carrier detection (phenotypic expression) in an autosomal-recessively transmitted disorder has been observed in various human disorders including sickle cell anemia (18), galactosemia (19), and phenylketonuria (20). This phenomenon has necessitated revision of the concepts of dominant and recessive inheritance (21) such that a third pattern, viz., intermediate, is now recognized. The evidence presented in this report would suggest that intermediate inheritance is the pattern for chick muscular dystrophy.

Summary. Chickens heterozygous for muscular dystrophy have been compared with homozygous normals and homozygous dystrophics with respect to plasma enzyme activity, pectoral muscle function, and muscle histology. Results for the heterozygote include increased mean plasma CPK and GOT, diminished pectoral muscle function, and alterations in muscle histology suggestive of dystrophy. These findings are discussed in relation to other hereditary animal dystrophies and human muscular dystrophy.

1. Julian, L. M. and Asmundson, U. S., in "Muscular Dystrophy in Man and Aminals." Courne G. H. and Golarz, M. N., eds. Karger, Basel, 1963.

2. Farrell, P. M., Eyerman, E. L., and Tureen, L. L., Ann. N. Y. Acad. Sci. 138, 102 (1966).

3. Chung, C. S., Morton, N. E., and Peters, H. A. Am. J. Human Genet. 12, 52 (1960).

4. Milhorat, A. T. and Goldstone, L., J. Am. Med. Assoc. 194, 130 (1965).

5. Hughes, B. P., Brit. Med. J. 2, 963 (1962).

6. Kelly, S., Kelly, W. D., and Swift, H. L., Am. J. Clin. Pathol. 45, 377 (1966).

7. Sigma Technical Bulletin No 661, Sigma Chem. Co., St. Louis, Missouri.

8. Sigma Technical Bulletin No. 505, Sigma Chem. Co., St. Louis, Missouri.

9. Asmundson, U. S., Kratzer, F. H., and Julian, L. M., Ann. N. Y. Acad. Sci. 138, 49 (1966)

10. Tureen, L. L., Farrell, P. M., and Cova, R. R., Proc. Soc. Exptl. Biol. Med. 119, 28 (1965).

11. Holloday, T. A., Asmundson, U. S., and Julian, L. M., Enzymol. Biol. Clin. 5, 209 (1965).

12. Homburger, F., Nixon, C. W., Eppenberger, M., and Baker, J. R., Ann. N. Y. Acad. Sci. 138, 14 (1966).

13. Hosenfeld, D. J., Wiesmann, U., and Richterich, R., Enzymol. Biol. Clin. 2, 246 (1962).

14. Eppenberger, M., Nixon, C. W., Baker, J. R., and Homburger, F., Proc. Soc. Exptl. Biol. Med. 117, 465 (1964).

15. Schapira, F., Schapira, G., and Dreyfus, J. C., Compt. Rend. Sci. Paris 245, 754 (1957).

16. Walton, J. N., Brit. Med. J. 1, 1271 (1964).

17. Emery, A. E. H., Lancet 1, 1126 (1963).

18. Callendar, S. T. and O'Brien, J. R. P., in "Biochemical Disorders in Human Disease," Thompson, R. H. S. and King, E. J., eds., Academic Press, New York, 1957.

19. Holzel, A. and Komrower, G. M., Am. J. Human Genet. 10, 53 (1958).

20. Knox, W. E., Messinger, E. C., Am. J. Human Genet. 1958, 10, 53 (1958).

21. Lenz, W., "Medical Genetics," Lanzl, E. F., Translator. Univ. of Chicago Press, Chicago, Illinois, 1963.

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