Plasma Aldolase and Glutamic-Oxaloacetic Transaminase Activities in Inherited Muscular Dystrophy of Domestic Chicken.* (24825)

C. E. Cornelius, G. R. J. Law, L. M. Julian and V. S. Asmundson
School of Veterinary Medicine and Dept. of Poultry Husbandry, Davis, Calif.

Increased serum aldolase(1,2) and glutamic-oxaloacetic transaminase(3) (SGO-T) activities have been observed in human progressive muscular dystrophy. Elevations in serum aldolase activity have also been reported in hepatitis(4,5), prostatic carcinoma(6,7), and acute myocardial infarction(8) of man. Only recently, enzyme studies in mice which show a hereditary muscular dystrophy, revealed increases in serum aldolase activity (9). These studies indicated an unchanged muscle aldolase activity in reference to non-collagen protein nitrogen of the tissue. Succinoxidase activity of dystrophic muscle tissue was not significantly affected, while cytochrome oxidase and cathepsin activities were markedly increased(9). In human patients with muscular dystrophy, it has been reported that serum GO-T activity increases while muscle GO-T activity, in reference to non-collagen protein nitrogen, remains unchanged(10). Weinstock *et al.*(9) have tentatively concluded that in human clinical muscular dystrophy or atrophy from neurotomy, glycolytic enzyme activity generally decreases and respiratory enzyme activity is not affected. In contrast to this, respiratory enzyme activity tends to increase and glycolytic enzyme activity remains relatively unchanged in muscular dystrophy induced by Vit. E deficiency or the hereditary type observed in inbred mice. Myocardial infarction(11) and hepatic necrosis in dogs(12) has recently been found associated with increased SGO-T activity in serum. It has also been observed that in bovine(13) and ovine(13,14) white muscle disease, a marked increase in SGO-T activity was present. Kuttler and Marble(14) observed increases in SGO-T activity in experimental white muscle disease of sheep as early as 12 days prior to development of clinical signs of the disease. They observed that levels of SGO-T roughly paralleled severity of clinical symptoms. Normal values concerning plasma and tissue activity of GO-T in chickens and other domestic animals have been reported by Cornelius *et al.* (12). Asmundson and Julian(15) described an inherited muscular abnormality in New Hampshire chickens. Gross and microscopic pathology of muscles appeared comparable to some of the muscular dystrophies of man. They reported that the affected birds exhibited wider breasts and shorter bones than normal individuals and were homozygous for autosomal recessive gene. Continuing with these investigations in chickens, the present study was undertaken to determine the following: 1. Plasma aldolase and GO-T activities in normal homozygous and heterozygous non-dystrophic male and female New Hampshire chickens; 2. Plasma aldolase and GO-T activities during clinical onset of inherited muscular dystrophy in New Hampshire chickens of strain 3 of the Univ. of California flock; and 3. GO-T activities in tissues of normal and strain 3 dystrophic New Hampshire chickens.

Materials and methods. Individual heparinized blood samples were collected by venipuncture of right jugular vein and centrifuged soon after bleeding. Plasma GO-T activity was measured by the method of Cabaud *et al.* (16). Tissue glutamic oxaloacetic transaminase activity was determined by modification of procedure of Tonhazy *et al.*(17). Samples were incubated 20 minutes at 25°C rather than at 10 minutes at 37°C as in original procedure. Accordingly, both plasma and tissue samples were incubated similarly. The technic for assaying glutamic-oxaloacetic transaminase activity is based upon transfer of the alpha-amino group of aspartic acid to alpha-ketoglutaric acid. The oxaloacetate which results from transamination is converted to pyruvic acid by aniline citrate. A pyruvate-dinitrophenylhydrazone is prepared,

* This study was supported in part by research grant from NIH.

TABLE I. Mean Plasma Aldolase Activities, as Expressed in "Dihydroxyacetone" Units, in Dystrophic and Normal Birds of Both Sexes Bled at 4 Successive Ages.

	Dystrophic birds				Normal birds	
	Group A		Group N*			
Age (days)	♂	♀	♂	♀	♂	♀
24	4.776 (5)†	1.807 (5)	.980 (3)	1.325 (7)	.955 (5)	.957 (5)
34	5.636 "	4.198 "	2.118 "	3.334 "	.427 "	.646 "
42	2.844 "	1.730 "	2.477 "	3.350 (8)	.741 "	.678 "
50	3.581 "	2.014 "	2.328 "	2.799 (7)	.728 "	.789 "
Avg	4.209	2.437	1.976	2.702	.713	.768

* This group appeared clinically normal between 23-26 days of age.
† Figures in parentheses refer to No. of samples in mean.

extracted with toluene, and measured colorimetrically at 490 mμ. Enzymatic activity is expressed as μg of pyruvic acid liberated in 20 minutes at 25°C, ml of serum or mg of dry tissue. Plasma aldolase was determined by standardization with dihydroxyacetone as recommended by Friedman and Lapan(18). Aldolase liberates both glyceraldehyde-3-phosphate and dihydroxyacetone phosphate from fructose-1, 6-diphosphate. Both trioses exhibit similar color development in a 60 minute period(19). Since both dihydroxyacetone and glyceraldehyde produce identical chromogenic derivates (methylglyoxal-2, 4-dinitrophenylosazone and pyruvic-2, 4-dinitrophenylhydrazone), either triose may be used as secondary standard in place of the more complex analysis for alkali-labile phosphate previously used. In our study, aldolase activity is expressed in terms of "dihydroxyacetone units" in which 1 unit equals amount of color developed by 0.01 mg dihydroxyacetone under the experimental conditions. All data were subjected to statistical analysis by means of analysis of variance. Transformations were made of aldolase data to correct for heterogeneity of variance(20).

Results. Plasma aldolase activities: A summary of results of individual aldolase determinations in plasma from both normal and dystrophic chickens is presented in Table I. Group A consisted of birds which exhibited clinical signs of muscular dystrophy at onset of study, while birds in Group N were free from abnormality at that time. All birds appearing clinically normal exhibited similar normal aldolase activities. Group N, however, showed muscular abnormality at second sampling, associated with increased serum aldolase activity. The data indicate that a pronounced elevation of aldolase activity occurs in all birds exhibiting dystrophy. Group N exhibited no aldolase elevations 7 days prior to occurrence of clinical symptoms of dystrophy. Elevation in activity of this enzyme in plasma in dystrophic birds may represent a change in cellular permeability and/or necrosis. Detailed statistical analyses of variances indicated that in dystrophic birds, age had a highly significant effect, $P<0.01$ (Table I).

Furthermore, interaction between groups and ages, and groups and sexes in dystrophic birds was also significant at same level of probability. This would indicate, therefore, that age and sex of birds as well as presence of muscular abnormality influenced the level of aldolase activity in plasma.

Additional data were obtained from 24 dystrophic and 31 normal birds varying in age from 64 to 365 days. Considering these birds and those reported in Table I, aldolase activities were 2.620 and .652 "dihydroxyacetone" units respectively. Differences due to age were significant with a reduction in aldolase activity in birds over 7 weeks old. It was of special interest to note that when data from older birds were examined by analysis of variance, the effects of sex showed no statistical significance. Considering all aldolase data, the major factors affecting plasma activity were the phenotype and age of bird. The data showed that effect of sex was a factor during early growth of birds but not after birds had passed the age of 7 weeks.

Plasma GO-T activities. Mean plasma GO-T activities in normal and dystrophic birds are presented in Table II. Dystrophic

birds exhibited elevated serum GO-T activities. This difference between dystrophic and normal birds was highly significant ($P<0.01$) but no source of variation, main effect or interaction, was significant except in the case of sex difference in the year-old group of dystrophic birds. Furthermore, analysis of data for each of the four 365-day-old groups, normal and dystrophic (Table II) showed sex genotype interaction, significant at the 5% level of probability. It should be noted that fewer determinations of plasma GO-T activity were performed on samples from young birds than in aldolase activity determinations. Had more ages been represented in the plasma GO-T series, an effect of age and/or sex might have been found. This possibility is substantiated by the observation that correlation between plasma aldolase and GO-T activities was highly significant ($r = 0.69$). This calculation was based upon data from 39 samples in which both aldolase and GO-T activities were determined. Both sexes and groups of birds at 3 different ages were represented.

Tissue GO-T activities. GO-T activities in various tissues of mature birds are presented in Table III. In nearly all tissues analyzed, GO-T activity/mg dry tissue was higher in dystrophic birds. These data show considerable variation in activity of this enzyme among tissues. Statistical analysis revealed that variations among tissues, as well as differences between normal and dystrophic birds were highly significant ($P = <.01$). This does not mean that GO-T activity was higher in all tissues of dystrophic birds but only in the majority of tissues. Higher activities in these tissues were not just a chance occurrence. Much more data are needed to obtain statistically valid inferences for each tissue of dystrophic and normal birds. No other source of variation, including comparison of parenchymous and muscular tissues, significantly influenced this variability. Muscle GO-T activity in human dystrophic muscles was shown to be unchanged when referred to non-collagen protein tissue nitrogen or lower than in normal muscles when related to basis of wet or dry tissue(10) as in our investigation. It was of special interest that muscle GO-T activity/mg dry tissue of dystrophic chickens was in comparison significantly elevated. Since nearly all tissues of dystrophic birds revealed elevated GO-T activities in this investigation, interpretation concerning increased or decreased transaminase activity in dystrophic muscle should be viewed with uncertainty. Relationship of tissue transaminase activity in chickens to protein anabolism and catabolism as reported by Beaton *et al.*(21) in rats merits further investigation.

TABLE II. Mean Plasma GO-T Activities in Dystrophic and Normal Birds of Both Sexes at Various Ages (Pyruvate Liberated/ml Plasma).

Age, days	Dystrophic birds ♂	Dystrophic birds ♀	Normal birds ♂	Normal birds ♀
28	648.7 (6)*	649.1 (6)	226.7(16)	238.2(17)
50	542.0 (5)†	295.5 (5)†	170.0 (5)†	233.6 (5)†
87	837.8(13)	771.0(12)	169.5 (5)†	263.7 (4)†
189	443.9 (5)†	260.0 (5)†		
365	1475.7(10)	819.3(15)	346.4(14)	299.9(12)
Avg	789.6	559.0	228.2	258.9

* Figures in parentheses refer to No. of samples in mean.

† Both plasma GO-T and aldolase activities determined in these samples.

TABLE III. Mean GO-T Activities in Tissues of 12- to 18-Month-Old Dystrophic and Normal Chickens (Pyruvate Liberated/ml Plasma).

Tissue	Dystrophic ♂ (2)*	Dystrophic ♀ (4)	Normal ♂ (3)	Normal ♀ (4)
Superficial pectoral	134	45	89	71
Deep pectoral	114	62	80	76
Biceps	83	123	90	91
Gastrocnemius	228	230	138	103
Gizzard	184	159	129	127
Heart	547	594	453	486
Liver	178	321	181	205
Kidney	243	307	202	228
Spleen	97	89	78	68
Brain	144	234	223	203

* No. of birds in parentheses.

Summary. New Hampshire chickens with onset of inherited muscular dystrophy exhibited elevated plasma aldolase and glutamic oxaloacetic-transaminase (GO-T) activities. Age and sex of birds influenced plasma aldolase activity in both normal and dystrophic groups. GO-T activities varied in different tissues and were greater in a majority of tissues examined from dystrophic birds when compared to normal birds.

1. Dreyfus, J. C., Schapira, G., Schapira, F., *J. Clin. Invest.*, 1954, v33, 794.

2. Schapira, G., Dreyfus, J. C., Schapira, F., *Semaine hop. Paris,* 1953, v29, 1917.

3. White, A. A., Hess, W. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 54.

4. Sibley, J. A., *Proc. Staff Meet., Mayo Clin.,* 1954, v29, 591.

5. Bruns, F., Jacobs, W., *Klin. Wchnschr.,* 1954, v32, 1041.

6. Baker, R., Govan, D., *Cancer Res.,* 1953, v13, 141.

7. Baker, R., Govan, D., Huffler, J., Cason, J., *J. Clin. Endocrinol.,* 1953, v13, 383.

8. Volk, B. W., Losner, S., Aronson, S. M., Lew, H., *Am. J. M. Sci.,* 1956, v232, 38.

9. Weinstock, I. M., Epstein, S., Milhorat, A. T., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 272.

10. Dreyfus, J. C., Schapira, G., Schapira, F. S., Demos, O., *Clin. Chem. Acta,* 1956, v1, 434.

11. Siegel, A., Bing, R. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 604.

12. Cornelius, C. E., Bishop, J., Switzer, J., Rhode, F. A., *Cornell Vet.,* 1959, v49, 116.

13. Blincoe, C., Dye, W. B., *J. An. Sci.,* 1958, v17, 224.

14. Kuttler, K. L., Marble, D. W., *Am. J. Vet. R.,* 1958, v19, 632.

15. Asmundson, V. S., Julian, L. M., *J. Hered.,* 1956, v47, 248.

16. Cabaud, P., Leeper, R., Wroblewski, F., *Tech. Bull. Reg. Med. Technol.,* 1956, v26, 230.

17. Tonhazy, N. H., White, N. G., Umbreit, W. W., *Arch. Biochem.,* 1950, v28, 36.

18. Friedman, M. M., Lapan, M. D., *J. Lab. Clin. Med.,* 1958, v51, 745.

19. Beck, W. S., *J. Biol. Chem.,* 1955, v212, 847.

20. Snedecor, G. W., 5th Edit., Iowa State College Press, 1956.

21. Beaton, G. H., Curry, D. M., Veen, M. J., *Arch. Biochem. and Biophys.,* 1957, v70, 288.

Received January 20, 1959. P.S.E.B.M., 1959, v101.

Electrolyte Alterations in Human Plasma and Erythrocytes Associated With Chronic Alcoholism.* (24826)

GEORGE BARLOW AND RICHARD L. WOOTEN† (Introduced by R. R. Overman)
Clin. Physiology Laboratories and Alcoholic Rehabilitation Center, University of Tennessee College of Medicine, Memphis

Few of the reports in the literature dealing with alcohol intoxication and alcoholism as a medical problem are concerned with its influence on water and electrolyte metabolism. Investigations have been confined to studies of response of normal individuals imbibing alcohol under control conditions, in specific amounts for short periods. Nicholson and Taylor(1) in one of the earliest reports showed that, despite a water diuresis after alcohol administration, Na, K and Cl ions were retained. Only 50% of the retained K could be accounted for in the extracellular fluid of these individuals. Subsequent studies(2,3) have largely complemented the earlier work except that the mode of action of alcohol in production of diuresis has been more accurately delineated(3,4). More recently reports have appeared concerning effects of alcohol on salt and water metabolism in the dog(5,6). Apparently the nature of response to alcohol in this animal is dose-linked. The present report concerns plasma and erythrocyte alterations in 64 human *chronic* alcoholics associated with malnutrition and generalized debilitation.

Methods. The 64 subjects (56 male, 8 female) were chronic alcoholic patients at the Alcoholic Rehabilitation Center. Their ages ranged from 25-67 years with a mean age of 44. All were in poor physical condition with approximately one-half having previous delirium tremens. Most showed signs of poor nutritional background. Ninety % had been drinking heavily for at least 10 years; most had a history of 20 years of alcoholism. While it was difficult to obtain reliable information from the patients, we were reasonably certain that the patients had been continuously drinking from 3 days to 12 months before admission. Beverages consumed consisted of one

* Supported in part by Grant-in-Aid from Wyeth Labs.

† Division of Medicine, Univ. of Tennessee College of Medicine.