pyrimidine analogues of thiamine are very active in growth inhibition of microorganisms shown to be resistant to both amethopterin and purine antagonists.

It is a reported clinical observation (9) that patients suffering from Hodgkin's disease, leukemia, and alimentary tract carcinoma, all have two to three times normal amounts of thiamine in their sera. The exact meaning of this fact is unclear, yet it may well represent a reflection of increased metabolic activity associated with a concomitant increased mobilization and/or utilization of thiamine. Hypothetically, this may fit in with the interpretation that certain tumor cells may mobilize and require more thiamine than normal cells.

The increasing reports in the literature regarding the use of various enzymes as antitumor agents lends credence to the hypothesis that a "thiaminase" may be the modality of action of various seafood extracts as oncostatic or oncocidal agents. Further investigations utilizing thiamine analogues as well as degradation of thiamine in various model tumor systems are being conducted.

Summary. The DBA/2 mice infected with Friend leukemia virus and maintained on a thiamine-free diet showed no histologic evidence or clinical manifestations of leukemia compared with counterpart controls maintained on a normal diet. Aliquot groups originally thiamine-deprived and subsequently refed thiamine developed gross splenomegaly and histologic evidence of leukemia. Similarities in preparation of materials from shellfish extracts in experiments to produce a "thiaminase," ichthiamin, and those used in antineoplastic experiments suggest a correlation in mechanism of action: thiamine inhibition.

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Delay of Hereditary Muscular Dystrophy of the Chicken by Oxygen Therapy: Histology*† (32953)

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Hereditary muscular dystrophy in the chicken is a myopathy most easily characterized by the loss of functional ability of the breast muscles (1). Using the exhaustion test as a measure of this functional ability, we have consistently observed that dystrophic birds, from our stock, reach peak scores at about 2.5 to 3.5 weeks of age and then fall

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[1] N.I.H. Predoctoral Fellow, 7-F1-GM-20, 128-01A1.

rapidly off toward zero. By 5 weeks of age the mean score for any group of these birds is below 3. On the other hand, the score of normal control birds rises rapidly through the first 3–5 weeks and levels off at mean values between 15 and 20.

In a previous communication (2), it was reported that dystrophic chickens raised in a high oxygen environment show marked improvement in their functional ability when compared to control birds raised in the normal atmosphere. When removed from the high oxygen environment, these birds rapidly become dystrophic as judged by the exhaustion test. This paper describes the histology of dystrophic chicken muscle as it is affected by oxygen therapy.

Materials and Methods. Eggs from normal and dystrophic stock[2] were incubated under standard conditions until day 19. At that time, half of each group was transferred to an oxygen environment chamber where the eggs were hatched and where the chicks remained until they were sacrificed for histological examination. The other half of each group, which constituted the controls, were hatched in an incubator of standard design and under normal conditions.

The oxygen environment chamber was constructed of clear Plexiglass with a raised wire screen floor. Oxygen was administered via a hose from a tank outside of the chamber, and CO_2 was removed by means of a recirculating pump connected to a sodium hydroxide filter. Temperature was regulated by running water of the proper temperature through copper tubing on the floor of the chamber. Temperature and humidity, in both the chamber and the cage (conventional wire-type), which housed the control birds, were comparable throughout the experimental period. Food and water were available *ad libitum* to both groups. At no time during the experimental period was there any noticeable difference in daily activities amongst the groups of birds. The oxygen concentration in the chamber was maintained at 45 ± 5% from day 19 until the chicks were hatched, at which time it was raised to 70 ± 5% where it remained until the birds were sacrificed.

At the end of the 6-week experimental period, the chicks were sacrificed by injecting air into the heart. The breast muscles were examined for gross characteristics, and tissue samples were excised from the right anterior quarter of the superficial breast muscle parallel to the fiber direction for histological examination. These samples were immediately placed in 10% neutral formalin for 48 hours and were then routinely embedded in paraffin, sectioned, and stained with hemotoxylin and eosin.

Results and Discussion. The gross symptoms commonly observed for dystrophic muscle were present in all of the control birds with the inherited myopathy. The color was invariably dull, greyish-pink, and opaque. In most individuals, the muscle was hypertrophied and, in all cases, visible striations were seen running parallel to fiber orientation. The texture of the tissue was rubbery and tough. The surface, *in situ,* was always covered with a network of blood vessels.

Breast muscle from the normal control chickens was pale yellow in color and translucent. Its texture was soft and sticky to the touch, and white striations were absent. The surface network of blood vessels was not visible. No gross differences were detected between normal chickens raised in air and those raised in high oxygen, with the exception of size. The breast muscles of chickens raised in air (both normal and dystrophic) were larger than those raised in oxygen.

Breast muscle from dystrophic chickens raised in high oxygen exhibited gross symptoms intermediate between normal muscle and muscle from dystrophic control birds. Coloration differences were apparent when compared to normal controls, but striations were absent, and the texture was more like normal than dystrophic muscle.

For microscopic examination, the prepared slides were given a code number and then scored for degree of cytological alteration, using a range of 0–3. A zero score indicated normal tissue, while a score of 1–3 indicated

[2] Dystrophic chicks are from our own stock, which was started from a flock maintained by Dr. Louis Pierro, Department of Animal Genetics, University of Connecticut. White leghorns served as normal controls.

TABLE I. Degree of Cytological Alteration in Normal and Dystrophic Chickens.

Untreated controls (21% O_2)		Experimental (70% O_2)	
Normal	Dystrophic	Normal	Dystrophic
0	2	0	1
0	2	0	0
0	3	0	0
0	2	0	1
0	3	0	0
0	3	0	1
	3		1
	2		1
	3		1

increasing degrees of degeneration. The results are presented in Table I.

All normal tissues were scored as zero. There was no discernible difference between those subjected to high oxygen and those raised in a normal atmosphere, with the exception that oxygen-treated birds had muscle fibers of smaller diameter. The fibers in both cases exhibited prominent cross-striations, narrow, oval-shaped nuclei (usually sarcolemmal), and were polygonal in cross section (Figs. 1 and 2).

Muscles from untreated dystrophic birds showed a high degree of fiber degeneration and were scored as 2 or 3. Characteristically, they exhibited a wide variation in fiber size. Large, hypertrophied fibers usually showed signs of degeneration, such as loss of striations, or focal areas of myolysis (Fig. 3). In addition, a large increase in the number of nuclei was observed. This appeared to be due both to an increase in sarcosomal nuclei, as well as from proliferating connective tissue, and from phagocytes (Figs. 3 and 4). Sarcosomal nuclei were usually rounded and vesicular, and occupied a more central location in the fiber. Arrangement of nuclei in rows was commonplace, particularly in hypertrophied fibers, and appeared to precede longitudinal fiber splitting. Large numbers of atrophied fibers were also evident as were apparently normal ones. Although signs of regeneration were seen, such as fiber budding, and myoblast activity, it was obvious that degenerative processes predominated over regenerative ones.

On the contrary, the muscle of dystrophic chicks raised in the high oxygen environment showed, in general, only minimal changes. Most fibers exhibited normal morphology. Visible degenerative changes were infrequent (Figs. 5 and 6). There was a noticeable increase, in most cases, in the number of muscle fiber nuclei when compared to normal controls. In addition to this increase in number, the morphology of the nuclei was more like those from dystrophic controls. However, it appeared that in treated birds, the regenerative effort was better able to compensate for the degenerative processes that were initiated by the genetic defect.

The significance of the network of blood vessels invariably seen on the surface of the breast muscles of dystrophic chicks is not known. This network of blood vessels had not been noticed previously, due to the fact that the chicks were killed by bleeding. Another difference seen in this study, involved the muscle capillaries. In all normal muscle so far observed, numerous capillaries can be seen as long files of erythrocytes (Fig. 1). Such files are rarely seen in dystrophic muscle either in control birds or in those treated with oxygen. Rather, erythrocytes are seen singly or in small clumps between muscle fibers giving the impression that many of the capillaries are not filled.

This difference is of interest since, in the event of a circulatory disturbance, delivery of oxygen (among other things) would likely be affected. Although circulatory defects commonly associated with muscular dystrophy have generally been regarded as secondary to the primary myopathy, the contribution of these, if any, to the progress of degeneration is not known. Malfunction of a control mechanism, or any other functional impairment of the microcirculation in muscle, could result in localized anoxia. White muscle in the chicken, which has little myoglobin for oxygen storage (3), becomes dystrophic earlier and to a much greater degree than red muscle fibers. In addition, white fibers are larger than red fibers and apepar to have a poorer blood supply. Consequently, white muscle

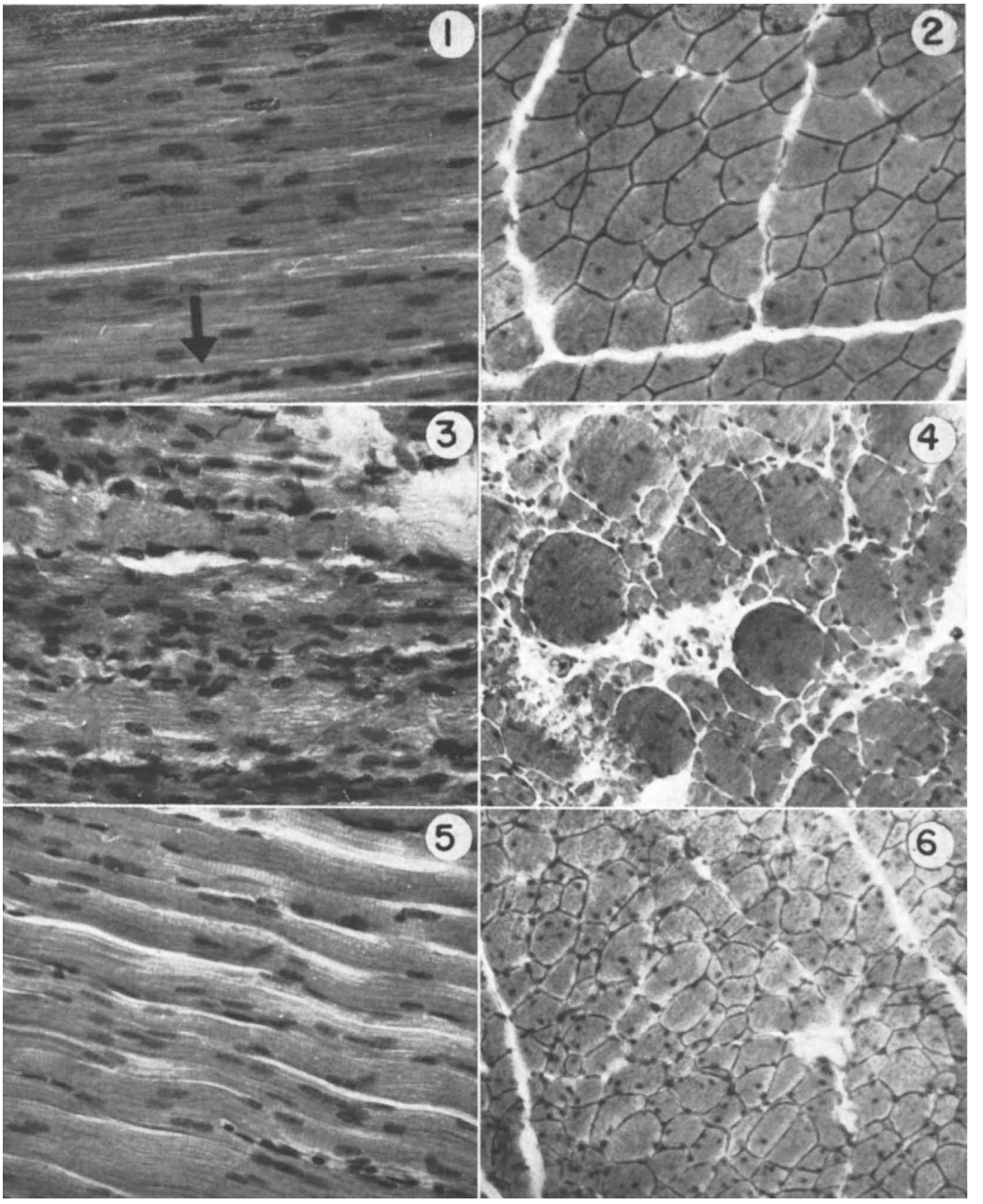

FIG. 1. (l.s. × 1280). Breast muscle of normal chicken raised in 21% oxygen. Arrow points to capillary.

FIG. 2. (c.s. × 390). Source of muscle same as Fig. 1.

FIG. 3. (l.s. × 1280). Breast muscle of chicken with muscular dystrophy raised in 21% oxygen.

FIG. 4. (c.s. × 390). Source of muscle same as Fig. 3.

FIG. 5. (l.s. × 1280). Breast muscle of chicken with muscular dystrophy raised in 70 ± 5% oxygen.

FIG. 6. (c.s. × 390). Source of muscle same as Fig. 5.

fibers would be at a severe disadvantage in the event of a circulatory disturbance. The delivery of oxygen and other blood borne materials to intracellular locations may be further impaired by hypertrophy of injured fibers. A disturbance in the microcirculation in human patients with Duchenne muscular dystrophy has been suggested by Demos (4) as a result of observed abnormalities in the peripheral circulation time.

The biochemical effects of high oxygen tension, on dystrophic chicken muscle, are not known. It is possible that oxygen therapy is effective due to an alteration in the microcirculation. We have not yet determined how long these beneficial effects of therapy can be maintained. It may be concluded, however, from this study and from functional data previously reported (2), that continuous high oxygen therapy does retard the progress of hereditary muscular dystrophy of the chicken during early stages of the myopathy.

Summary. Fertile eggs from normal and genetically-dystrophic chickens were placed in an environment chamber on day 19 of development. The atmosphere contained 45% oxygen until the chicks hatched, at which time it was raised to 70%. The chickens were maintained in the high oxygen atmosphere until 6 weeks of age when they were sacrificed for histological examination. The breast muscles of oxygen-treated dystrophic chickens underwent only minimal cytological changes when compared to both untreated normal and dystrophic controls. Breast muscles of oxygen-treated normal chickens showed no changes. It is concluded that continuous treatment in a high oxygen environment retards the progress of the hereditary myopathy.

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Rat Tissue DPNH–Cytochrome *c* Reductase Activity in Altered Thyroid States* (32954)

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Changes in the activities of many enzymes have been studied in a variety of tissues after alterations in thyroid states. The effect of thyroidectomy on smooth muscle, however, has not been extensively investigated. Barker (1) found the succinoxidase activity of the uterus to be quite variable after thyroidectomy and after thyroxine treatment. Since enzyme complex DPNH–cytochrome *c* reductase studies have been limited to liver with respect to thyroid function (2), this present investigation is concerned with effects of thyroidectomy and thyroxine treatment on liver, heart, bladder, and uterus with respect to the activity of this enzyme. Effects of thyroidectomy on the activity of DPNH–cytochrome *c* reductase and DNA content in the estrogen-stimulated uterus are also reported.

Materials and Methods. Female albino Sprague-Dawley rats weighing between 150–200 gm were ovariectomized and divided into five groups: sham thyroidectomized, thyroidectomized, thyroidectomized given daily injections of L-thyroxine (100 μg/kg of body wt.) for 2 weeks, sham thyroidectomized and thyroidectomized given daily injections of estradiol 17β (2.1 μg/kg of body wt.) for 3 days. Animals were fed *ad libitum* and were given 1% $CaCl_2$ as drinking water for 1 month during the development of the hypothyroid state and before thyroxine and estrogens were

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