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Delay of Hereditary Muscular Dystrophy of the Chicken by Oxygen Therapy.*† (31336)

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The occurrence of a hereditary muscular dystrophy in the chicken with features similar to that of man has been reported by Asmundson and Julian(1). This muscle abnormality prevents the birds from raising their wings or returning to a standing position when placed on their backs on a flat surface. Chung *et al*(2) concluded that the exhaustion number, which is the number of times a bird can be placed on its back until it can no longer rise, is the best means of early detection of the abnormality.

Many authors have reported biochemical and histochemical alterations of dystrophic tissues. Our laboratory has shown that in 8-week-old, genetically-dystrophic chicks, reduced myoglobin (myoglobin without oxygen) in muscle tissue extracts is approximately 35% higher relative to normal controls. Schapira and Dreyfus(3) have reported a diminution in oxygen consumption in human dystrophic muscle. They further report that many female carriers show an apparent acceleration of the peripheral circulation time. These facts suggested to us the possibility that muscle cell death may be initiated by chronic oxygen starvation.

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Materials and methods. In a preliminary experiment (Experiment I, Table I), 5 chicks with hereditary muscular dystrophy were placed in a high oxygen environment at 1 day of age and maintained there until 28 days of age. Five dystrophic controls from the same hatch and same parents were maintained in an adjacent cage, but without added oxygen. A standard chick starter ration and water were fed to both groups *ad libitum*.

Both cages were about the same size, having approximately 7 square feet of floor space per cage. The cage, which housed the oxygen-maintained birds, was constructed of wood with the top and front covered with polyethylene. In order to allow free circulation of the oxygen, which was admitted *via* a hose from an oxygen tank, and to allow escape of carbon dioxide, the cage was perforated along the bottom. The cage containing the dystrophic controls was a conventional wire battery brooder cage. In both cages, all birds had free access to food and water. There was no noticeable difference in activity between the oxygen-maintained birds and the controls at any time.

Unfortunately, during this preliminary experiment, the oxygen concentration inside of the experimental cage was not monitored. However, oxygen was admitted at a constant rate of 1 liter/cu ft/min throughout the 28-day period of the experiment. Temperatures were generally the same in both cages, although occasional differences were noted.

TABLE I. Comparison of the Exhaustion Numbers of Dystrophic Chicks Following Removal from an Oxygen Enriched Environment with Those of Non-Treated, Dystrophic and Normal Controls.

Experiment I

	Days of age				
	28	29	31	40	42
	Oxygen-treated dystrophics				
Mean	10.7	16.3	17.0	4.7	0.7
Range	8–12	10–19	9–26	0–12	0–1
No. birds	3	3	3	3	3
	Dystrophic controls				
Mean	2.0	1.6	1.6	0.8	0.4
Range	0–7	0–5	0–4	0–2	0–1
No. birds	5	5	5	5	5

Experiment II

	Days of age								
	33	35	37	39	41	43	47	51	54
	Oxygen-treated dystrophics								
Mean	7.0	10.8	12.8	8.1	6.8	4.7	4.1	1.7	1.2
Range	1–11	7–17	8–24	0–16	0–15	0–11	0–8	0–7	0–7
No. birds	13	12	11	10	10	10	10	10	10
	Dystrophics hatched and treated in oxygen								
Mean	10.3	12.0	13.4	10.0	8.9	8.5	6.9	4.0	3.5
Range	5–16	5–20	7–24	3–26	2–15	2–21	3–21	0–20	0–20
No. birds	9	9	9	9	9	8	7	7	7
	Dystrophic controls								
Mean	2.3	2.4	2.4	2.4	2.2	1.9	0.9	0.7	0.5
Range	0–9	0–10	0–11	0–12	0–10	0–8	0–8	0–7	0–5
No. birds	16	16	16	16	16	16	16	16	16
	Normal controls								
Mean	—	11.2	12.0	12.3	13.3	14.3	15.1	16.0	14.9
Range	—	7–16	7–17	8–19	8–20	10–20	12–20	11–22	9–20
No. birds	—	22	22	22	22	14	14	14	14

These differences were slight and it is doubtful that they influenced the results.

In Experiment II (Table I), 13 day-old dystrophy-chicks and 12 dystrophy-embryonated eggs at the 19th day of incubation were placed in the high oxygen environment and maintained there for 33 days under conditions identical to those cited for Exp. I. Of the 12 embryonated eggs, all but 3 hatched. Thus a total of 22 dystrophics were maintained in the oxygen environment. Sixteen dystrophic controls and 22 normal controls were placed in separate cages, again without added oxygen. Food and water were fed under the same conditions as in Exp. I. In Exp. II, oxygen concentration was monitored daily with a Beckman 777 analyzer and maintained at 70 ± 5% throughout the 33-day period of the experiment.

Following each experimental period, oxygen therapy was terminated, but all other conditions were maintained. At this time, and at the ages indicated in Table I, the dystrophic chickens were tested for the clinical onset of the myopathy by application of the exhaustion test. As described earlier, the exhaustion test consists of recording the number of times a bird can return to standing position after being placed on its back.

Results. Upon removal from the high oxygen environment, some of the treatment birds had difficulty in breathing and tired easily. In Exp. I (Table I), 2 birds died within minutes of being removed from the oxygen. However, 3 birds survived and their data, as well as those from the dystrophic controls, are presented in Table I.

The dystrophic controls already exhibit the

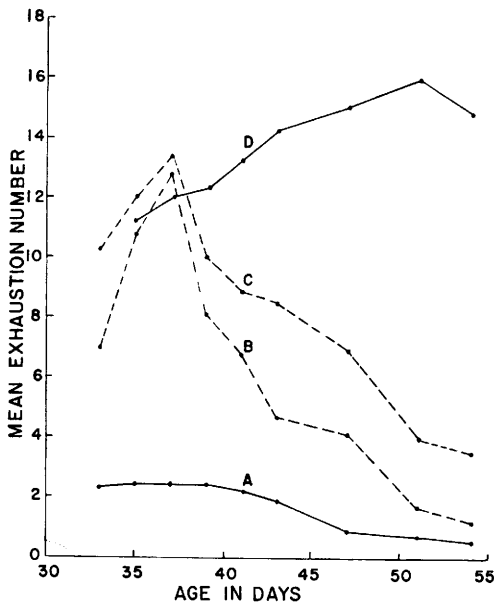

FIG. 1. Comparison of mean exhaustion numbers from Exp. II. Exhaustion numbers were determined starting at 33 days of age when the 2 oxygen-treated groups (curves B and C) were removed from oxygen. (A): Non-treated dystrophic controls; (B): oxygen-treated dystrophics; (C): dystrophics hatched and treated in oxygen; (D): non-treated normal controls.

clinical state of the disease by 28 days of age as evidenced by both the range and mean exhaustion numbers. In contrast, the oxygen-maintained birds had both mean and range values comparable to normal birds for at least the 4-day period immediately following removal from oxygen. By 40 days of age, however, most of the oxygen-treated birds were unable to flip onto their feet, and by 42 days of age, were comparable with the dystrophic controls.

The data from Exp. II (Table I) closely parallel the results of the first experiment. Again the oxygen-maintained birds remained comparable to the normal controls for several days following removal from oxygen, but by 51 days of age, both the mean and range values approximated the dystrophic controls. The same results are apparent for the chicks which were hatched in oxygen with comparable values somewhat higher than the birds placed in oxygen at time of hatching. Fig. 1 shows the data from Exp. II in graphic form.

Discussion. The apparent improvement in ability of the oxygen-maintained birds to right themselves when placed on their backs during the 4 or 5 days immediately following removal from oxygen is probably a reflection of an adjustment to the normal atmosphere. In a high oxygen environment, the synthesis of red blood cells and hemoglobin is depressed (4). Thus an animal placed in a normal atmosphere after continuous exposure to high oxygen would have a lower-than-normal oxygen carrying capacity. As the oxygen carrying capacity was improved by synthesis of hemoglobin and red blood cells, there would be a parallel and progressive increase in strength.

In examining the range of the dystrophic chicks which were hatched in oxygen, it will be noticed that the upper range at 54 days of age is still 20. This was due to a single chick (as is implied by the mean) which at 63 days of age was still flipping over 20 times. This bird decreased to 0, indicating that it was a true dystrophic. Whether this particular bird was naturally slow in expressing the clinical state of the disease or was responding exceptionally well to the oxygen, is not known. Probably a combination of both resulted in its unusually long delay in becoming dystrophic. However, it should be pointed out that never have any of our untreated dystrophics been able to regain a standing position 20 times, and only a very few are able to flip over at all beyond 5 weeks of age.

It would appear from these results that oxygen therapy may either alleviate or retard the symptoms of hereditary muscular dystrophy of the chicken. Obviously many more types of tests have to be employed, both biochemical and histological, before the full value of oxygen treatment will be known.

It is not possible to provide an explanation for these results based on these limited experimental data. That exposure to high oxygen environments will result in elevated tissue oxygen concentrations is well documented. Weiss *et al*(4) have shown that when chicks 2-7 weeks of age are exposed to 100% oxygen at one atmosphere the arterial oxygen tension increases by 300% over that of normal controls, while pH and arterial carbon dioxide

tension are unaffected. Ackerman *et al*(5) directly measured, with miniature polarographic electrodes, significant increases in muscle oxygen tension in rabbits following inhalation of 100% oxygen. Likewise, Bean has reported(6) that cerebral tissue oxygen is raised to about twice its normal level when breathing oxygen at atmospheric pressure.

If sufficient oxygen is unavailable to the muscle cell, particularly during periods of high metabolic activity such as rapid growth (which apparently is when muscular dystrophy is most progressive) or during muscular exercise, then it is possible that a transient anoxia could damage a structural membrane.

Zierler(7) found that among other agents absence of oxygen would accelerate the efflux of aldolase from rat diaphragm. Substitution of carbon monoxide for oxygen also causes an increase in serum creatine-kinase(3) indicating an increased muscle cell permeability. White muscle fibers have little or no myoglobin(8) which is commonly believed to act as a storehouse for oxygen. It might then be expected that the white fiber would be more susceptible to metabolic injury in the event of insufficient oxygen. It is interesting in this connection that the white muscle fiber of the chicken is much more severely affected and becomes dystrophic prior to the red muscle fiber.

Herrmann *et al*(9), and Morgan and Herrmann(10), have provided evidence that there is a retardation of muscle maturation in the dystrophic chick. It is possible that such a delay in development might be connected with a deficient oxygen supply to the muscle cell.

If oxygen therapy proves to be of any lasting or substantial benefit to chickens with hereditary muscular dystrophy, it may give some insight into the genetic defect at the molecular level which in turn may shed some light on similar myopathies of other animals including man.

Summary. Two groups of chickens with hereditary muscular dystrophy were subjected to a high oxygen environment for 28 and 33 days respectively. Exhaustion numbers show that following removal from the oxygen enriched environment, the dystrophic birds were more like normal controls than dystrophic controls and remained that way for 4 to 5 days. However, they subsequently exhibited the clinical signs of muscular dystrophy.

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