Effect of Arginine Deficiency on Nutritional Muscular Dystrophy in the Chick.* (25986)

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Nutritional muscular dystrophy in the chick is of complex dietary etiology. The disease, in chicks, is affected by dietary amounts of Vit. E, methionine, cystine, and selenium. Addition to the deficient diet of either Vit. E or one of the sulfur-containing amino acids completely prevents the condition(1,2). Supplementing the basal diet with selenium results in partial prevention of muscular dystrophy (3,4). During investigations as to whether the effects of the sulfur amino acids were specific for preventing the disease, deficiencies of other essential amino acids were studied to determine effect on development of muscular dystrophy. A study of the effect of an arginine deficiency disclosed an interesting interaction between dietary arginine level and development of muscular dystrophy. The following experiments show that with an arginine deficiency nutritional muscular dystrophy did not develop in the chicks, even though the diet was also deficient in Vit. E and the sulfur-containing amino acids.

Experimental. Male and female White Plymouth Rock chicks hatched from eggs produced by a flock of hens maintained on a Vit. E-low diet were used in all experiments. They were housed in thermostatically - controlled batteries with wire mesh floors. The basal diet used was as follows: casein, 20.00; cerelose, 66.56; cellulose, 3.00; stripped lard, 4.00; mineral mixture, 5.49; vitamin mixture, 0.95. The vitamin mixture has been described by Morrison *et al.*(5) and contains no Vit. E. The mineral mixture supplied the following in g/kg of diet: $CaHPO_4$, 21.51; $CaCO_3$, 14.92; KH_2PO_4, 8.67; NaCl, 6.00; $MgCO_3$, 1.42; $FePO_4 \cdot 4H_2O$, 2.65; $MnCl_2 \cdot 4H_2O$, 0.463; KI, 0.0026; $Cu(C_2H_5O_2)_2$, 0.0119; $ZnCl_2$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.001; $NaMoO_4 \cdot 2H_2O$, 0.0083; KCl, 1.5.

Muscular dystrophy was readily diagnosed by appearance of grossly visible white striations running in the direction of muscle fibers on the breast of the chicken, as described by Dam *et al.*(1). The chicks were sacrificed at the end of each experiment and the skin removed from the breast to make the final assessment of incidence and severity of muscular dystrophy.

Experiment 1 was designed to study effects of deficiencies of arginine, glycine and methionine on muscular dystrophy. These amino acids were added to the basal diet alone, and in all possible combinations. The effects of Vit. E were also studied with the basal diet and in a diet with arginine and glycine added. The levels of amino acids used were chosen because these were the levels present in a previous basal diet(3) containing casein and gelatin as protein sources, which had been the standard diet for study of muscular dystrophy in this laboratory. The results of this experiment are included in Table I. Muscular dystrophy was observed only in the chicks receiving arginine without added Vit. E or methionine. No muscular dystrophy was observed in the chicks receiving the basal diet or this diet supplemented with glycine.

Since growth of the chicks receiving the arginine deficient diets was considerably less than in chicks receiving diets adequate in arginine, a second experiment was conducted in which one group of chicks receiving the basal diet plus arginine was pair-fed with the basal group to eliminate the difference in size of the chicks receiving these 2 diets. In other treatments, chicks receiving additions of arginine alone, arginine plus methionine, and arginine plus Vit. E were fed these diets *ad libitum*. The results of this experiment (Table II) confirm those of the previous experiment in that supplemental arginine was found to be essential for development of muscular dystrophy even when the growth of those receiving added arginine was restricted by limited feed intake.

* This work was aided by grants from Muscular Dystrophy Assn. of America, Inc., Nutrition Foundation, Inc., and Distillation Products Industries, who also supplied the stripped lard and *d*-α-tocopheryl acetate used in the studies.

TABLE I. Amino Acid Deficiencies of Casein and Muscular Dystrophy in the Chick.

Treatment	Avg wt, 5 wk, g	Muscular dystrophy
Basal	209 (16)*	0/16†
+ .4% DL-methionine	172 (7)	0/17
+ 1.01% L-arginine HCl	378 (4)	11/14
+ 2.74% glycine	262 (17)	0/17
+ .4% methionine + 1.01% L-arginine HCl	455 (14)	0/14
+ .4% DL-methionine + 2.74% glycine	218 (17)	0/17
+ 1.01% L-arginine HCl + 2.74% glycine	416 (16)	12/16
+ *idem* + .4% DL-methionine	419 (8)	0/ 8
+ 80 mg/kg d-α-tocopheryl acetate	206 (16)	0/16
+ 1.01% L-arginine HCl + 2.74% glycine + 80 mg/kg d-α-tocopheryl acetate	376 (15)	0/15

* No. of survivors.
† Expressed as No. of chicks showing symptoms (numerator) over No. of chicks examined (denominator).

Vit. E or methionine supplementation prevented development of muscular dystrophy.

A third experiment was conducted to determine whether higher levels of arginine would increase the severity of the disease in chicks. The basal diet was supplemented with levels of 1.01, 2.08 and 4.15% arginine HCl. The arginine-supplemented groups were all fed the same amount of feed as the basal group after 5 days of age. Results of this experiment (Table III) confirmed the previous

TABLE II. Effect of Arginine on Development of Muscular Dystrophy.

Treatment	Avg wt, 4 wk, g	Muscular dystrophy	Feed/ chick
Basal	216 (17)*	0/17§	420
+ 1.01% arginine HCl†	248 (18)	17/18	392
+ *Idem*‡	298 (19)	18/19	489
+ 1.01% arginine HCl + .4% DL-methionine	380 (20)	0/20	565
+ 1.01% L-arginine HCl + 80 mg/kg d-α-tocopheryl acetate	304 (19)	0/19	510

* No. of survivors.
† Fed same amount of feed as basal after one wk of age.
‡ *Ad libitum* fed.
§ See footnote †, Table I.

2 experiments in that supplemental arginine was necessary for development of muscular dystrophy in young chicks. Severity of the muscular dystrophy was measured by means of an index. Affected muscles were given a score of from 1 to 10 depending on amount of breast muscle affected by the lesions. A score of 10 indicated the most extensive muscular degeneration. Based on this scoring system, higher levels of arginine had no significant effect on the severity of the dystrophy.

To obtain further evidence on a possible relationship between arginine level, growth and muscular dystrophy, another experiment was conducted in which several groups of chicks were given the arginine-deficient diet for 5 weeks, whereupon some of the groups were then switched to the diet with adequate arginine supplementation. Within 2 weeks thereafter (when the chicks were 7 weeks of age) all chicks receiving arginine showed muscular dystrophy while those not receiving arginine still did not appear to be affected by the disease. Feed intake was equalized in this experiment again to eliminate large differences in body size.

TABLE III. Effect of Various Levels of Arginine on Development of Muscular Dystrophy.

Treatment	Avg wt, 5 wk, g	Muscular dystrophy	Severity index
Basal	204	0/20*	—
+ 1.01% L-arginine HCl	261	17/20	7.0
+ 2.08%	270	19/19	8.4
+ 4.15%	261	18/20	8.0

* See footnote †, Table I.

Discussion. These experiments indicate another dietary variable of importance in nutritional muscular dystrophy in the chick. Development of nutritional muscular dystrophy in chicks appears to depend not only on levels of Vit. E and sulfur-amino acids, but also upon level of arginine in the diet.

The specific reason for the relationship between arginine deficiency and muscular dystrophy observed in this study is not known. Differences in rate of growth did not appear to be important, since muscular dystrophy was equally severe when growth of the chicks given adequate arginine was limited by restricted feeding.

A possible explanation of the results obtained in these experiments may lie in the effect of the sulfur-amino acids on muscular dystrophy. Since arginine was the first limiting amino acid in the basal diet, utilization of the remaining amino acids of the diet for protein synthesis was depressed. More methionine and cystine would then become available to the animal for use in other functions, one of which may be involved in prevention of muscular dystrophy. If this is the case, deficiencies of essential amino acids other than arginine may have the same dystrophy-depressing effects.

These experiments also provide evidence that the sulfur amino acid effect is not a nonspecific effect, observed only because of a deficiency of an essential amino acid. As opposed to the effect of methionine a deficiency of the essential amino acid arginine did not result in muscular dystrophy in chicks fed a Vit. E deficient diet.

Summary. Nutritional muscular dystrophy in the chick did not occur when an arginine deficient diet was fed even though the diet was low in Vit. E and sulfur amino acids. When arginine was included in the basal diet, a high incidence of muscular dystrophy was observed by 5 weeks of age, even when growth was restricted in the chicks receiving adequate arginine by paired feeding with the basal group.

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Received June 29, 1960. P.S.E.B.M., 1960, v104.

Growth of Human Cancer Cells (HEP 2) in Newborn Rats.* (25987)

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Transplantation of antigenically foreign tissue is usually followed by a prompt host reaction resulting in rejection of the transplant unless special conditions prevail. Conditions which permit growth of heterologous tissues include special implantation sites such as the brain and the anterior chamber of the eye(1,2), "conditioning" of recipient animal by agents such as cortisone and x-ray which cause a general depression of defense mechanisms(3), and use of an embryonic or newborn recipient such as the chick embryo(4). Each of these methods has been used successfully for the heterologous growth of human cancer cells. More recently it has been reported that the human epidermoid cancer cell line HEp #3 will grow in rats inoculated *in utero* (5) in newborn unconditioned hamsters†, and in newborn unconditioned mice(6). The present report describes growth of another human epidermoid carcinoma cell line (HEp #2) in normal newborn rats.

Methods. The HEp #2 cell line of Toolan (7) was grown in monolayer tissue cultures, harvested with trypsin and washed once with Gey's salt solution. The cells were resuspended in Gey's solution, counted in a hemocytometer, and adjusted to 8 to 10 million cells per ml. Non-inbred albino rats less than 24 hours old were inoculated intravenously in the facial or submental veins with 0.25 ml of this suspension. Inoculation failures were discarded from this study. Rats which died within 2 to 3 days after inoculation were also excluded. Litter mate controls were inoculated with an equal volume of Gey's salt solution.

* This study supported by research grants from Nat. Cancer Inst., U.S.P.H.S., and Phoebe Waterman Fund.

† Personal communication.