

2, 3, 4 were observed only when large amounts of radioactive  $\text{Co}^{57}$   $\text{B}_{12}$  were added. Since the peaks consist of mixtures of proteins, identification of the specific proteins binding  $\text{Co}^{57}$   $\text{B}_{12}$  cannot be delineated.

**Summary.** A method was described for the separation of vit  $\text{B}_{12}$  binders in normal serum on a  $15 \times 4$  cm DEAE- cellulose column by stepwise elution with 0.005 M pH 8.0, 0.0175 M pH 6.3, 0.04 M pH 5.9, 0.1 M pH 5.8 and 0.4 M pH 5.2 sodium phosphate buffers. Two radioactive peaks appeared when 300  $\mu\text{g}$   $\text{Co}^{57}$   $\text{B}_{12}$  per ml of serum had been added *in vitro*. With the addition of 2,000  $\mu\text{g}$ /ml of  $\text{Co}^{57}$   $\text{B}_{12}$  to the serum, 5 radioactive peaks

appeared.

1. Hall, C. A., Finkler, A. E., J. Lab. & Clin. Med., 1965, v65, 459.
2. Hom, B., Olesen, H., Lous, P., *ibid.*, 1966, v68, 958.
3. Retief, F. P., Gottlieb, C. W., Kochwa, S., Pratt, P. W., Herbert, V., Blood, 1967, v29, 501.
4. Adams, E. C., Yoder, J. M., Free, A. H., in Serum Proteins and the Dysproteinemias, F. W. Sunderman & F. W. Sunderman, Jr., ed., Lippincott, Philadelphia, 1964, 148.
5. Peterson, E. A., Sober, H. A., in Methods in Enzymology, S. P. Colowick & N. O. Kaplan, ed., Academic Press, New York, 1962, v5, 3.

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### Serotonin Antagonist Increases Longevity in Mice With Hereditary Muscular Dystrophy.\* (32512)

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The occurrence of hereditary muscular dystrophy in an inbred strain of mice was first reported by Michelson *et al*(1), and intensive research on these animals since that time has indicated that, in addition to histologic changes, a number of biochemical, histochemical, and physiologic differences exist in their skeletal muscle. Several recent investigations have implicated a high content of the biogenic amine, serotonin (5-hydroxytryptamine), with this myopathy and with other skeletal muscle defects. Gordon and Dowben(2), while studying the distribution of adrenaline and noradrenaline in mice with hereditary muscular dystrophy, found approximately twice the concentration of serotonin in the spleen of afflicted mice as in normal mice, and its presence was thought possibly to represent a physiologic response to enhanced sympathetic activity or to a general disorder in amine metabolism. When normal mice were given daily injections of serotonin for over 3 weeks, focal lesions, characterized by hyaline sarcoplasmic degeneration and myofibrillar destruction, developed throughout the skeletal mus-

culature(3). Studies of other endocrine or humoral factors in dystrophic mice are rare, but Alger and Boccabella(4) found that sexual maturity was delayed in both sexes.

The present study was undertaken to establish if treatment with a specific serotonin antagonist would modify in a short period of time such characteristics as body weight and weights of several endocrine organs and the kidneys, and if treatment with such an agent over extended periods of time would influence survival of afflicted mice and the progressive development of the myopathy.

**Materials and methods.** Strain 129/Re male and female mice were obtained from Jackson Memorial Laboratories, and animals with normal (*DyDy* or *Dydy*) and dystrophic (*dydy*) genotypes were divided into control and treated groups. All animals, which were 4 to 6 weeks old on arrival from the supplier, were maintained until the 7th week, when treatment was begun, to standardize the beginning of the experimental period. Groups of male and female mice of normal and dystrophic phenotypes were injected intraperitoneally daily with either 2.0 or 5.0 mg/kg body weight of the serotonin antagonist, 1-methyl-

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TABLE I. Comparison of Normal and Dystrophic Mice After 3 Weeks of Treatment with Serotonin Antagonist, Methysergide Maleate (Organ Weight = mg/100 g Body Wt).

	No. of mice	Body wt	Gonad wt	Pituitary wt	Adrenal wt
Normal males					
Controls	5	27.92 $\pm$ .75	814.2 $\pm$ 53.11	5.19 $\pm$ .21	11.43 $\pm$ .58
Treated (2 mg/kg)	5	28.40 $\pm$ .79	804.2 $\pm$ 48.25	5.25 $\pm$ .11	12.33 $\pm$ .37
Dystrophic males					
Controls	5	18.50 $\pm$ .79	1019.2 $\pm$ 71.33*	6.93 $\pm$ .35*	17.53 $\pm$ 1.82*
Treated (2 mg/kg)	5	20.20 $\pm$ .70	1012.5 $\pm$ 70.88*	7.09 $\pm$ .32*	15.94 $\pm$ 1.30*
Normal males					
Controls	9	31.00 $\pm$ .81	719.8 $\pm$ 50.40	6.30 $\pm$ .32	
Treated (5 mg/kg)	8	27.68 $\pm$ .90	765.9 $\pm$ 54.71	6.47 $\pm$ .32	
Normal females					
Controls	10	20.40 $\pm$ .69	34.20 $\pm$ 2.39	8.39 $\pm$ .49	
Treated (2 mg/kg)	10	20.13 $\pm$ .80	37.42 $\pm$ 2.62	8.19 $\pm$ .50	
Dystrophic females					
Controls	8	16.02 $\pm$ .44	34.20 $\pm$ 2.48	10.53 $\pm$ .64*	
Treated (2 mg/kg)	10	14.93 $\pm$ .70	42.61 $\pm$ 3.02†	8.90 $\pm$ .72*	

\*  $p < .01$ , compared to either normal control or treated mice.†  $p = .05$ , compared to dystrophic female control mice.

D-lysergic acid butanolamide bimalate (methysergide bimalate, UML-491, SAN-SERT Sandoz) for 3 weeks.

At autopsy, following etherization, the pituitary gland, testes, ovaries, ventral prostate, preputial gland, adrenals, and kidneys were fixed in Bouin's solution; after fixation, they were removed, dissected free of extraneous tissue, dried carefully on paper towels and weighed on a Roller-Smith torsion balance.

Other groups of normal and dystrophic mice of both sexes were caged individually, and water intake was carefully measured daily for at least one week subsequent to arrival of animals. When 7 weeks old, they were divided into control and treatment groups according to body weight. From calculations of daily fluid intake and body weight a solution of methysergide bimalate was prepared in a concentration so that each mouse received approximately 2.0 mg/kg body weight per day in their drinking water. Fluctuations in fluid intake and in body weight were continually evaluated and adjustments of drug concentration were made when needed to maintain constant dosage. Mice receiving this treatment were not killed or autopsied, but instead were kept until death to determine the effect of the drug on their maximal survival time.

All animals were caged in a temperature ( $25 \pm 1^\circ\text{C}$ ) and light (6 AM to 8 PM CST)

regulated room and fed *ad libitum* a standard mouse diet,<sup>†</sup> which was finely ground and placed in dishes for dystrophic mice.

**Results. Short term treatment.** Methysergide bimalate, when given daily in 2 or 5 mg/kg doses for 3 weeks, caused no significant changes in body weight, weight of pituitary, adrenal, preputial, and ventral prostate glands, or of kidneys. Only the organ weights with statistically significant variations are in Table I. Ovaries of dystrophic mice treated with serotonin antagonist (2 mg/kg) were enlarged significantly as compared to those of dystrophic control mice; ovarian weights of dystrophic control mice were identical to those of the normal control group. Average ovarian and testicular weights in normal mice were increased following treatment with methysergide (2 mg/kg in females and 5 mg/kg in males) as compared to control gonads, but the increment was statistically insignificant. Although fluctuations in pituitary weight seemed directional, (an increase in males and decrease in females following treatment), changes were statistically insignificant. Pituitary and adrenal glands of male and female dystrophic mice were significantly larger than those of normal animals (Table I), when organ weight was expressed as mg/100 g body weight. Absolute weights of pituitary and adrenal glands,

† Purina Mouse Breeder Chow.

TABLE II. Survival in Weeks ( $\pm$  S.E.) of Dystrophic Mice Treated with Methysergide Maleate, a Serotonin Antagonist.

Controls		Treated		% Increase in sur- vival	
Groups	No.	Age	No.	Age	
Females					
I	4	23.4 $\pm$ 1.9	4	42.9 $\pm$ 3.3*	83.3
II	6	25.6 $\pm$ 2.0	12	50.1 $\pm$ 5.2	95.7
III	5	22.8 $\pm$ 2.0	7	45.1 $\pm$ 5.2	97.8
Group avg	15	24.1 $\pm$ 2.0	23	47.3 $\pm$ 4.9	96.3
Males					
I	4	20.7 $\pm$ 1.5	4	41.6 $\pm$ 3.8	101.0
II	12	17.9 $\pm$ 1.4	10	32.9 $\pm$ 2.7	83.8
III	8	17.0 $\pm$ 2.1	4	33.0 $\pm$ 3.2	94.1
Group avg	24	18.1 $\pm$ 1.7	18	34.9 $\pm$ 3.1	92.8
Oldest surviving mouse					
Female	30.3		60.3		
Male	23.0		44.7		

\* Statistical analyses, using the Student *t* test, for significant differences of the means of control and treated mice in each group yielded *p* values of less than .01.

which are not shown on Table I, but which can be calculated from data presented, did not vary significantly between normal and dystrophic mice.

**Long term treatment.** Male and female dystrophic mice, divided into 3 groups (Table II) necessarily as a result of their availability from the supplier, were given methysergide bimalate in their drinking water, and records of their survival time indicated that this treatment greatly prolonged their life. Twenty-five percent (6) of the untreated males died by the end of the 10th week, and none survived beyond the age of 23 weeks; 28% (5) of the treated males died between the 20th and 25th week of age, while only one of this group survived for 44.7 weeks (Fig. 1).

Both control and treated dystrophic female mice outlived comparable male groups. Only one control female mouse died prior to the 15th week; the longest survival in this group was 30.3 weeks. Among the treated females, no deaths were recorded prior to the 30th week, and the oldest treated mouse lived to 60.3 weeks of age (Fig. 1).

The mean survival times for all groups of dystrophic mice are shown in Table II.

Body weights, which were recorded begin-

ning with the 8th week, gradually increased during the treatment period in control and treated groups (Fig. 2). At 8 weeks of age the average weight of control dystrophic males was  $17.44 \pm 0.58$  (S.E.) g, while that of control dystrophic females was  $13.67 \pm 0.68$  g. The average weight of comparable treated groups at this age was  $17.52 \pm 0.67$  g (males) and  $13.56 \pm 0.85$  g (females). Maximum weights of control males and females were 19.4 and 18.0 g, while those of treated animals were 24.0 and 21.6 g, respectively. A significant weight gain in females occurred after 5 to 6 weeks of treatment (13 to 14 weeks old) with the serotonin antagonist and

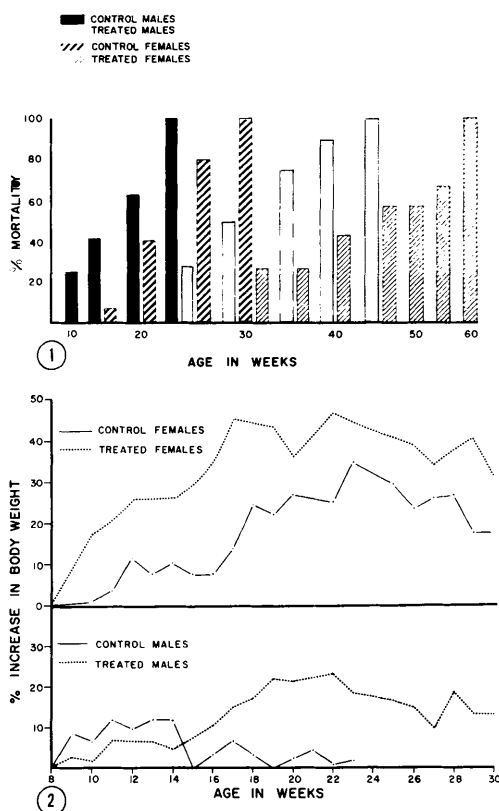


FIG. 1. Comparison of time sequence of deaths (expressed as cumulative % mortality) between control and methysergide treated groups of male and female dystrophic mice.

FIG. 2. Weekly fluctuations in body weights of control and methysergide-treated groups of male and female dystrophic mice. Since control groups did not survive beyond 30 weeks of age (see Fig. 1), changes are not shown beyond that time for all groups. Percentages were calculated from body weights at 8 weeks of age.

was maintained during the entire period prior to the death of all control mice.

There was no significant difference in body weight of control and treated dystrophic male mice during the first 14 weeks of age; the precipitous decline in control weight at 15 weeks of age (Fig. 2) followed deaths of 3 mice, who previously had shown the greatest weight gain. No significant increase in body weights of the remaining 15 control mice occurred during subsequent weeks. A statistically significant difference between body weights of treated and control males was observed during the 17th week of age and is reflected in the percent increase at that time (Fig. 2).

**Discussion.** Large numbers of tissue mast cells(5), which contain the biogenic amines, histamine and serotonin, and high concentrations of histamine in skin(6) and muscles (7), and of serotonin in the spleen of mice with hereditary muscular dystrophy(2) suggest a possible interrelationship between the biogenic amines and the development of the myopathy. Serotonin and histamine will induce histologic changes, such as sarcoplasmic necrosis, myofibrillar degeneration, and central rowing of muscle nuclei in skeletal muscle of normal mice(3,8). Results of the present study indicate that a specific serotonin antagonist, methysergide bimaleate, can prolong the life span of afflicted mice, irrespective of sex. However, untreated and treated females outlived comparable groups of males (Table I). Treated mice gained more weight, which indicates a greater intake, or better assimilation, of food and were in a better physical state as evidenced by smoother, shinier coats and clearer eyes than untreated dystrophic animals.

Survival time of dystrophic mice apparently can be greatly influenced by factors such as the type of available diet. Since the myopathy affects the jaw musculature as well as other skeletal muscle, standard mouse pellet diet was ground finely to facilitate feeding during the experiments in this report; seemingly, availability of ground food significantly increased the life span of both male and female mice (average age at death = 23.9 and 18.5 weeks, respectively; Table II), as compared to the results of Coleman and West(9). They

found that the average survival time of male and female dystrophic mice fed on the same pelleted, but unground, diet was 9.1 and 13.4 weeks, respectively, and that special high protein and lipid diets extended the growth period, decreased incidence of coagulation necrosis in skeletal muscle, and increased life span up to 26 weeks of age. Hall *et al*(10) found that, after parabiosis between a normal and dystrophic mouse, which allowed for intervascular nutriment exchange, life expectancy was increased to an average of 24 weeks in males and 25 weeks in females, as compared to 8 to 10 weeks in single dystrophic mice. Conflicting results show no prolongation of survival by parabiosis(11). Dowben(12), while studying the effects of 17  $\alpha$ -ethyl-19-nortestosterone and creatine synthesis in dystrophic mice, recorded a median survival time of 22 weeks for the control group and 33 weeks for treated animals. The longevity of dystrophic animals fed on a ground standard diet and treated with serotonin antagonist (Table II) exceeded that in the cited studies and closely compares to the mean attained age of 42 weeks obtained by Dowben *et al* (13) after feeding a vitamin enriched diet and administering anabolic androgenic steroids.

Short term treatment, *i.e.*, 2 or 5 mg/kg methysergide daily for 3 weeks, had little effect on normal and dystrophic mice of either sex. The lower dosage caused a significant increase in ovarian weight in dystrophic mice, and a noticeable, but statistically insignificant, increase in testis weight occurred after treatment with the high dose of serotonin antagonist. Interestingly, Alger and Boccabella (4) found delayed sexual maturity in both male and female dystrophic mice and associated it with a deficit in circulating androgen or estrogen. Since serotonin does inhibit ovulation, ovarian weight, and luteinization, delays the time of vaginal opening in immature, gonadotrophin-treated rats(14) and depresses testis weight in mice(15), and since the serotonin antagonist caused ovarian enlargement in dystrophic mice, delayed sexual maturity in affected animals possibly is related to abnormally high levels of circulating serotonin. Biochemical amine assays would be necessary to clarify this speculation.



West *et al*(16) have reported that symptoms of mouse muscular dystrophy were alleviated slightly after treating newborns daily with reserpine, a potent serotonin and catecholamine releasing agent. Exceedingly large concentrations of serotonin, which are directly related to the severity of the myopathy, have been demonstrated in the cerebrospinal fluid of human dystrophic patients(18).

Proportionally larger adrenal and pituitary glands were demonstrated in dystrophic mice as compared to normal animals, and short term treatment with the serotonin antagonist did not significantly affect the weight of these organs (Table I). Enlarged adrenal glands have been reported previously in these animals(4,10), and may be ascribed to the stress of the disease. Serotonin, incidentally, also can cause adrenal enlargement(17).

Administration of serotonin antagonist to mice with hereditary muscular dystrophy did not eliminate the symptoms of dystrophy, but instead, seemed to retard the progressive rate of their development, allowing for longer survival of affected animals. Further studies, possibly on fetal stages, may determine if serotonin is associated with the cause or with one of the many consequences of the myopathy.

**Summary.** Survival time was increased over 90% in mice with hereditary muscular dystrophy treated orally with a serotonin antagonist, methysergide bimalate. There was a concurrent increase in body weight in treated animals. Short term treatment for 3

weeks with the drug caused a significant enlargement of the ovaries of dystrophic mice, but had no effect on body weight or other endocrine organ weights of normal mice.

1. Michelson, A. M., Russell, E. S., Harman, P. J., *Proc. Nat. Acad. Sci.*, 1955, v41, 1079.
2. Gordon, P., Dowben, R. M., *Am. J. Physiol.*, 1966, v210, 728.
3. O'Steen, W. K., Barnard, J. L., Jr., Yates, R. D., *Anat. Rec.*, 1967, v157, 380.
4. Alger, E. A., Boccabella, A. V., *ibid.*, 1962, v142, 208.
5. O'Steen, W. K., Hrachovy, J. J., *Tex. Rpts. Biol. Med.*, 1962, v20, 70.
6. ———, *ibid.*, 1965, v23, 762.
7. Bois, P., *Am. J. Physiol.*, 1964, v206, 338.
8. O'Steen, W. K., *Experientia*, 1964, v20, 284.
9. Coleman, D. L., West, W. T., *J. Nutrition*, 1961, v73, 273.
10. Hall, C. E., Hall, O., Nevis, A. H., *Am. J. Physiol.*, 1959, v196, 110.
11. Pope, R. S., Murphy, E. D., *ibid.*, 1960, v199, 1097.
12. Dowben, R. M., *Nature*, 1959, v184, 1966.
13. Dowben, R. M., Zuckerman, L., Gordon, P., Sniderman, P., *Am. J. Physiol.*, 1964, v206, 1049.
14. O'Steen, W. K., *Endocrinology*, 1965, v77, 937.
15. ———, *Proc. Soc. Exp. Biol. & Med.*, 1963, v113, 161.
16. West, W. T., Meier, H., Hoag, W. G., *Ann. N. Y. Acad. Sci.*, 1966, v138, 4.
17. Benson, G. K., *Proc. Soc. Exp. Biol. & Med.*, 1960, v103, 132.
18. Misra, S. S., Bhargava, K. P., Singh, K. S. P., *J. Assoc. Physicians India*, 1965, v13, 337.

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### Mitotic Abnormalities Produced by Juglone in Ehrlich Ascites Tumor Cells.\* (32513)

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The cytological and biochemical effects of quinones have been studied in a variety of biological systems(1,2,7,8,9). We now have studied the cytological effects of 16 quinone compounds on Ehrlich ascites tumor cells *in vivo* and have found that one of them, juglone (5-hydroxy-1,4-naphthoquinone), produced

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